ARKIV FÖR KEMI Band 12 nr 43

Communicated 26 February 1958 by GÖSTA HÄGGQVIST and ERIK JORPES

Role of inanition in the reduced synthesis of chondroitinsulfuric acid in guinea pig rib cartilage in vitamin C deficiency

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With 7 figures in the text

Various histochemical investigations have suggested that vitamin C deficiency interferes with the production of the mucopolysaccharides of the connective tissue ground substance; possibly there is an accumulation of abnormal mucopolysaccharides (6, 10, 17, 18). Reddi and Norström (19) discovered that the *in vivo* incorporation of radiosulfate into rib cartilage chondroitinsulfuric acid (CSA) of scorbutic guinea pigs was about one-third of that in normal controls. Their animals were sacrificed after 21 days on the deficient diet.

In a recent investigation the present author administered radiosulfate to guinea pigs at different stages of vitamin C deficiency. The animals were sacrificed 48 hours later, viz. after 10, 17 and 23 days on the scorbutogenic ration. The radioactivities of various tissues and of CSA prepared from rib cartilage were determined. The incorporation of S^{35} into CSA was not markedly influenced at 10 or 17 days. The radioactivity of CSA from the 23 day scorbutic animals was about one-fourth of that of the normal controls. Concomitantly, however, there was a low radioactivity in all deficient tissues examined.

The reduced uptake of radiosulfate was not confined solely to cartilage. A reduction of S^{35} incorporation into CSA coincided with the great terminal loss of weight in the scorbutic animals. Hence it was not possible to establish either to what extent CSA synthesis was reduced in acute scurvy, or to what extent such a diminution was caused specifically by lack of ascorbic acid rather than inanition.

The present experiments were conducted in order to clarify the relationship between vitamin C deficiency and the formation of CSA. To evaluate the role of inanition, pair-feeding was adopted. Experiments were performed *in vivo* (experiment A) as well as *in vitro* (experiments B to D). The incorporation of S^{35} and C^{14} into CSA was studied with radiosulfate and generally labeled glucose, respectively, as precursors.

Material and methods

Animal techniques

For most of the experiments, rapidly growing guinea pigs of both sexes were used. They were kept in the laboratory for two weeks prior to the beginning of the actual experiments, by which time they weighed around 260 g. All animals received the same

vitamin C-free mixed basal diet *ad libitum*, and had free access to drinking water. A supplement of vitamin A, B_{12}, D. E, K and "P" (rutin) was also given. Synthetic ascorbic acid was administered during the two-week acclimatization period, and to all controls throughout the investigation. Some deficient animals, too, eventually received an ascorbic acid supplement (50 mg/day per os) and were allowed to recover; they are hereinafter referred to as recovery animals. Further details concerning the care of the animals will be found in a previous paper (8).

The following pair-feeding technique was employed. The animals were divided into pairs, matched in sex and weight, each animal being kept in a separate cage. The daily food consumption of the scorbutic animals was determined by weighing the ration, offered in excess, before and after feeding. The same amount of food that the scorbutic animals had ingested was given the next day to the respective controls.

For the *in vitro* experiments costal cartilage was taken from the guinea pigs or was obtained from young calves at a local slaughterhouse.

Tracer techniques

Carrier-free solutions of S^{35} sulfate and crystalline, generally labeled C^{14} glucose, specific activity 10 mc/m*M*, were obtained from Radiochemical Centre, Amersham, England. The radioactivities of intact CSA and of barium sulfate (experiment B) were determined at infinite thickness with a G-M tube after plating on brass discs, active area 1 cm^2 (8). CSA degradation products were plated with a surface area of 2.85 cm^2 on filter paper after conversion to barium carbonate or barium sulfate. The radioactivities were then measured in a gas flow counter (SC-16, Tracerlab, Inc., Boston, Mass.). At least 10,000 counts were registered.

Preparation and degradation of CSA

CSA was prepared by the method of Boström and Månsson (4, 5). In the C^{14} experiments non-radioactive glucose was added before dialysis, which was extended to 48 hours. The radioactivities of the sulfate group and of the uronic and hexosamine moieties were determined after decomposition of the polysaccharide. Samples of CSA were hydrolyzed for 5 hours in 4 *N* HCl at 110°C in sealed glass bulbs. The carbon dioxide evolved from carbon atom 6 in the uronic acid moiety was precipitated with saturated barium hydroxide containing 10 per cent barium chloride. The precipitate was collected under ether, washed with carbon dioxide-free water and plated on filter paper (21, 25). The rest of the hydrolysate was first evaporated to dryness in a desiccator over sodium hydroxide and then dissolved in a small quantity of water. Radiosulfate and hexosamines were thereafter separated on a column of Dowex 50, 2 × 40 cm. Essentially, the procedure of Gardell was followed (9). The radiosulfate emerged with the first few fractions. Fractions of approximately 10 ml were collected and aliquots were pipetted off for colorimetric determination of aminosugar (2, 7). The fractions containing galactosamine were pooled and evaporated to dryness. The hexosamine was then combusted and plated as barium carbonate on filter paper. To obviate contamination of radioacarbon samples with S^{35}, a silver wire mesh and a dish with potassium dichromate were enclosed in the combustion tube; furthermore the carbon dioxide evolved was led through hydrogen peroxide/sulfuric acid. The fractions emerging before the appearance of the aminosugars were pooled, and the radiosulfate precipitated as $BaSO_4$ without addition of carrier.

In vitro techniques

For the *in vitro* experiments the technique of Boström and Månsson was followed (5). The cartilage slices were incubated in Krebs–Ringer bicarbonate medium (26), KRB, with oxygen–carbon dioxide aeration. The samples were always preincubated for 1 hour before addition of the isotope. Incubation was then continued for 2 hours, unless otherwise stated (21).

The method (I) used in experiment C involves isolation of CSA from the cartilage slices, with subsequent determination of the radioactivity of the mucopolysaccharide or its hydrolytic products. A simplified, though reasonably exact method (II) was used in the other *in vitro* experiments (3, 20). This method does not require purification of CSA. The slices are freed from inorganic radiosulfate by a special washing procedure. The calf cartilage slices were sufficiently uniform to permit determination of their radioactivity directly on the dried specimens (experiment D). Since the shape of guinea pig rib slices, on the other hand, makes direct radio-assay inaccurate, these slices were weighed after drying, and refluxed for 8 hours with 6 *N* HCl at 100°C (experiment B). Tubes with ground glass joints and air condensers were used. Carrier sulfate was added to the hydrolysate and, after filtration, barium sulfate was precipitated. The precipitate was collected on filter paper, ignited, weighed and plated on brass discs. Radioactivities were calculated per mg dry cartilage.

Experiments and results

I. *In vivo studies*

A. *Incorporation of S^{35} and C^{14} into rib cartilage CSA*

This was studied in one scorbutic group, one recovery group, one pair-fed control group and one normal control group. The experiment embraced a total of 91 surviving guinea pigs, with a roughly even distribution among the four groups. Three scorbutic,

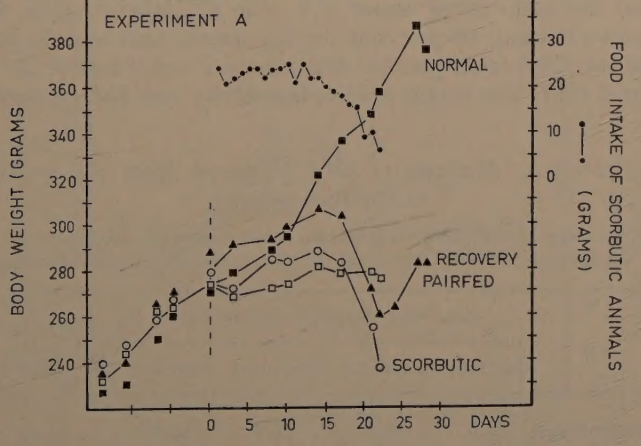

Fig. 1. Mean weight curves and food consumption of deficient animals, experiment A. Mean weight at beginning of experiment 278 g. Animals on the deficient diet began to eat less and to lose weight after appoximately 14 days. Administration of 50 mg vitamin C daily to scorbutic animals after 21 days caused rapid gain of weight-recovery group.

Table 1. Experiment A. Radioactivities of CSA degradation products expressed as cpm/2.85 cm^2 at infinite thickness.

The S^{35} label of CSA from scorbutic and pair-fed animals was about 28 per cent of normal; that from recovery animals, about 43 per cent of normal. The C^{14} label of scorbutic CSA moities was about 50 per cent above normal, and of pair-fed CSA moities about 100 per cent above normal. The ratios of uronic acid to hexosamine C^{14} label were about 2:1.

Group	C^{14} Uronic acid	C^{14} Hexosamine	S^{35} Sulfate group
Normal . . .	50	25	21,800
Scorbutic . .	83	29	5,700
Recovery . .	38	23	9,300
Pair-fed . . .	119	45	6,400

2 recovery and 4 pair-fed animals died during the experiment and were discarded. Each animal was given a single intraperitoneal injection of radiosulfate (0.16 μc/g) and C^{14} glucose (0.016 μc/g) in combination 24 hours prior to sacrifice. The scorbutic and the pair-fed guinea pigs were killed after 21 days together with half of the normal animals. The recovery animals were given a vitamin C supplement for 7 days after 21 days on the scorbutogenic diet, and were sacrificed together with the remaining normal controls after 28 days.

The mean weight curves of the groups and the food consumption of the deficient animals are shown in Fig. 1. CSA was prepared from the pooled rib cartilage of the respective groups. The polysaccharide was hydrolyzed and its degradation products recovered. The barium precipitates were plated on filter paper and the radioactivities determined in the flow counter. It will be seen from Table 1 that the S^{35} labels of CSA from the scorbutic group and from the pair-fed group were only about 28 per cent of normal. The radioactivity of the sulfate group of CSA from recovery animals was about 43 per cent of normal. The radioactivities due to C^{14} were higher in the hexosamine moieties, the ratio being about 2:1. The C^{14} label of CSA from scorbutic animals was above normal, 86 per cent for the uronic acid and 16 per cent for the aminosugar moiety. CSA from pair-fed animals had a still higher C^{14} label, in comparison to normal CSA. The uronic acid radioactivity was 138 per cent, the galactos-

Table 2. Experiment A. Analysis of CSA prepared from the pooled rib cartilage of the four groups.

No significant differences in chemical composition were observed. About 4 per cent of the total hexosamine content were due to the presence of glucosamine.

	Per cent of dry weight of CSA				Theoretical
	Normal	Scorbutic	Recovery	Pair-fed	$(C_{14}, O_{14}, H_{19}, NSNa_2)_n$
Hexosamine	24.5	29.0	23.3	25.0	35.59
Nitrogen . .	3.33	3.01	4.40	3.87	2.78
Sulfur . . .	4.81	5.30	4.65	4.93	6.37
Ash	18.2	17.4	16.5	14.7	28.22

amine radioactivity 80 per cent, above normal. The C^{14} label of CSA from recovery animals was slightly below normal.

Table 2 shows that there were no significant differences in chemical composition between the CSA preparations from the four groups of guinea pigs.

II. *In vitro studies*

B. *Incorporation of S^{35} during development of vitamin C deficiency*

In the following experiment the incorporation of radiosulfate into rib cartilage CSA was examined at weekly intervals during the progression of vitamin C deficiency. The primary aim was to see if the results of *in vitro* studies would duplicate those of the *in vivo* studies. A secondary aim was to elucidate the effects of addition of ascorbic acid to the KRB medium on the incorporation of S^{35} into CSA *in vitro*.

The incorporation of radiosulfate into CSA *in vitro* can be increased by adding glutamine to the incubating medium. This stimulation can be further enhanced by adding glucose as well (21). It was therefore of interest to find out, too, if scorbutic cartilage retained responsiveness to stimulation by glutamine and glucose.

A total number of 88 surviving animals was used. Two scorbutic, 1 recovery and 5 pair-fed animals and 1 normal control died during the experiment and were discarded. The animals were divided into 16 approximately equal groups. The mean weight curves and the food consumption of the deficient animals are presented in Fig. 2.

Rib cartilage was obtained from vitamin C-deficient guinea pigs after 7–8, 14–15, 21–22 days and from pair-fed animals after 21–22 days. Due to the limited capacity of the incubation apparatus the experiments had to be extended over two consecutive days. Cartilage was also taken from a group of recovery animals that received vitamin C per os for 7 days in a daily dose of 100 mg, after 22 days on the deficient ration. At each interval, cartilage was also removed from normal controls.

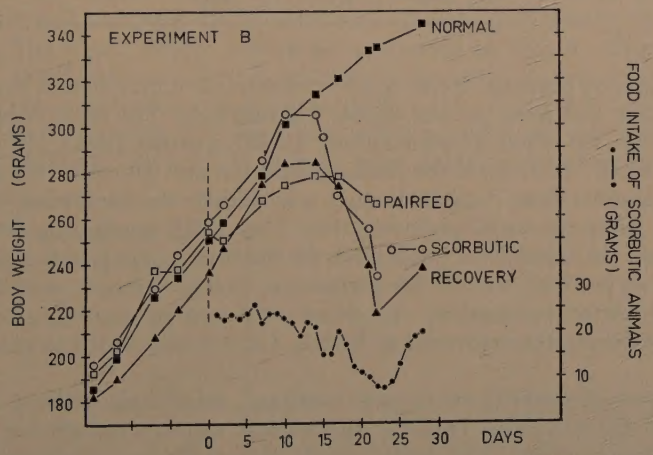

Fig. 2. Mean weight curves and food consumption of deficient animals, experiment B. Average weight at start of experiment around 245 g. Weight loss of deficient animals after about 15 days. Recovery animals were given 50 mg of ascorbic acid daily after 22 days on the scorbutogenic ration.

Table 3. Experiment B. Radioactivities due to S^{35} of cartilage incubated in KRB (—), in KRB with glutamine + glucose (GG) or in KRB with ascorbic acid (AA, figures refer to concentrations in mM/l).

The normal count for each day is arbitrarily taken as unity. Radioactivities of scorbutic and pair-fed cartilage were about 60 per cent below normal at 21–22 days. Normal S^{35} incorporation in recovery cartilage. Glutamine + glucose increased the radiosulfate uptake by about 40 per cent; ascorbic acid apparently had no influence thereon.

Days	Addition to KRB	Normal	Deficient and recovery	Pair-fed
7	—	1.00	1.00	
	GG	1.33	1.35	—
	AA 0.03	0.97	1.00	
8	—	1.00	1.14	
	GG	1.29	1.54	—
	AA 0.06	1.00	1.03	
14	—	1.00	1.02	
	GG	1.47	1.44	—
	AA 0.03	1.14	0.85	
15	—	1.00	1.04	
	GG	1.49	1.72	—
	AA 0.06	1.13	1.14	
21	—	1.00	0.33	0.41
	GG	1.29	0.43	0.60
	AA 0.03	0.92	0.35	0.44
22	—	1.00	0.44	0.46
	GG	1.58	0.59	0.56
	AA 0.06	1.25	0.45	0.47
22 + 7	—	1.00	1.05	
	GG	1.29	1.29	—
	AA 0.06	1.17	0.85	

The cartilaginous portions of the ribs were denuded of muscle and connective tissue as far as possible and were cut into pieces 1–2 mm long. The slices from one group of animals were pooled, then divided among 12 Erlenmeyer flasks (50 ml), each containing 5 ml KRB. To four of the flasks glutamine and glucose were added in combination; to another four, *l*-ascorbic acid was added; the remaining four contained no additions. After the usual preincubation, 1 ml KRB containing 20 μc S^{35} sulfate was added, and the incubation continued. In the final volume of 6 ml the concentration in milimols per liter was 0.1 for glutamine, 10.0 for glucose, and 0.03 or 0.06 for ascorbic acid. After incubation, the slices were treated according to method (II), and the radioisotope was recovered as $BaSO_4$. Carrier was added to give a precipitate of 150 mg.

The average radioactivity of normal cartilage, incubated without additions, was 300–400 cpm. In compiling Table 3, the normal count obtained for each day was arbitrarily taken as unity. The table reveals little influence of vitamin C deficiency on the synthesis of CSA during the first 15 days. At 21 and 22 days, however, the radioactivity of scorbutic cartilage was 55 to 65 per cent below normal. The uptake of S^{35} by cartilage from pair-fed controls was equally low. The response to glutamine

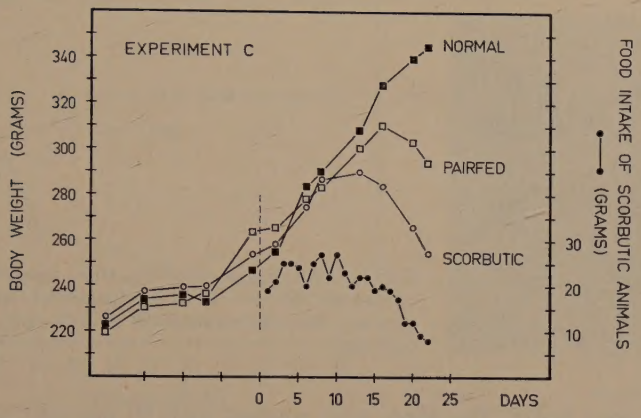

Fig. 3. Mean weight curves and food consumption of deficient animals, experiment C. Mean weight at start of experiment 255 g. Animals on the deficient diet lost weight after approximately 13 days.

and glucose consisted in an increase of S^{35} activity by about 40 per cent both in normal and in scorbutic and pair-fed cartilage. On the other hand, ascorbic acid in the concentrations used did not influence the S^{35} incorporation. Cartilage from recovery animals showed a normal incorporation of S^{35}.

C. *Utilization of C^{14} glucose for the synthesis of CSA*

In order to ascertain whether the results obtained with S^{35} sulfate were representative for the whole CSA molecule, C^{14} glucose was used in the following experiment.

The experiment was conducted with a total number of 52 surviving guinea pigs. Twenty-two of these were scorbutic; the remainder were divided equally among pair-fed and normal controls. Eight scorbutic animals died during the experiment (27 per cent). The mean weight curves are presented in Fig. 3. After 22 days the animals were sacrificed and the rib cartilage removed, as described above. The cartilage from one experimental group was pooled, then divided between two Erlenmeyer flasks, each containing 18 ml KRB without additions, After preincubation, 2 ml KRB containing 12.5 μc C^{14} glucose was added; incubation then continued

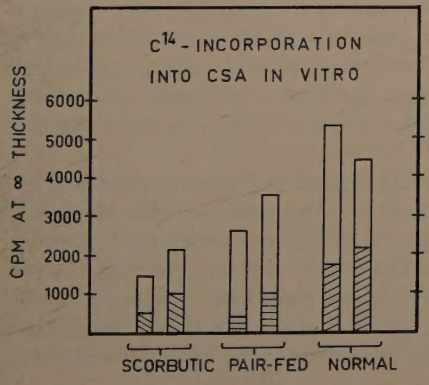

Fig. 4. Experiment C. Radioactivities of uronic acid (unfilled + striped) and hexosamine (striped) moities of CSA after incubation with C^{14} glucose. The radioactivity of CSA from scorbutic and pair-fed cartilage averaged 37 and 51 per cent, respectively, of that from normal cartilage.

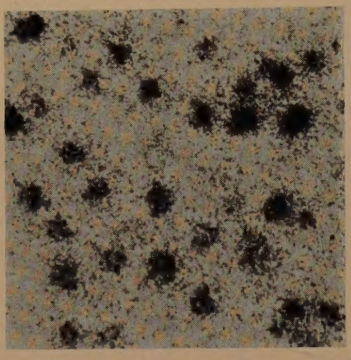

Fig. 5. Experiment C. Strip-film autoradiogram of 5 μ unstained section of rib cartilage slice, incubated with C^{14} glucose. The radioisotope is mainly confined to the chondrocytes.

and extended to 4 hours. The slices were washed with 10 per cent non-radioactive glucose, whereafter CSA was prepared from the slices—method (I). The radioactivities of the intact CSA samples were determined at infinite thickness. The following radioactivities were obtained: normal 6746, scorbutic 3193 and pair-fed 4215 cpm. With normal cartilage count rate as unity, the radioactivity of scorbutic cartilage was 0.47, and of pair-fed, 0.62.

Following this procedure the CSA samples were hydrolyzed. The hexuronic acid and hexosamine moieties were recovered as $BaCO_3$. The activities were corrected to infinite thickness. Fig. 4 shows the results. It will be seen that the activity of the uronic acid moiety was 2.5–3.5 times that of the amino sugar, except in one of the pair-fed samples where the corresponding factor was around 7.5. The radioactivity of the scorbutic and pair-fed cartilage CSA was reduced. Calculated from uronic acid radioactivity, it was found to be 64 per cent below normal in scorbutic and 36 per cent in pair-fed cartilage. Calculated from hexosamine radioactivity, the corresponding values were 62 and 61 per cent respectively.

Some cartilage slices were embedded in paraffin, sectioned and autoradiographed, using stripping film. It is evident from Fig. 5 that the radioisotope was localized mainly to the cartilage cells.

D. *Effect of ascorbic acid on the incorporation of S^{35} sulfate into CSA of normal calf rib cartilage*

In experiment B, no increase in uptake of S^{35} by scorbutic cartilage or by cartilage from normal or pair-fed controls was observed when the KRB medium contained ascorbic acid in concentrations equivalent to those in normal guinea pig whole blood. It was accordingly worth while investigating the effects of various concentrations of ascorbic acid on the S^{35} utilization or normal calf cartilage *in vitro*. Method (II) was used.

It is apparent from Fig. 6 that ascorbic acid inhibited the incorporation of S^{35} into CSA *in vitro*, when added to the KRB medium in concentrations well above the physiologic blood level. The inhibition amounted to about 80 per cent at a concentration of 1 millimol per liter, whether or not glutamine plus glucose had been added. Within the normal concentration range, the curve had a somewhat irregular course. The experiment was therefore repeated, using several concentrations of ascorbic acid within the physiologic range. Fig. 7 shows that there was already a definite

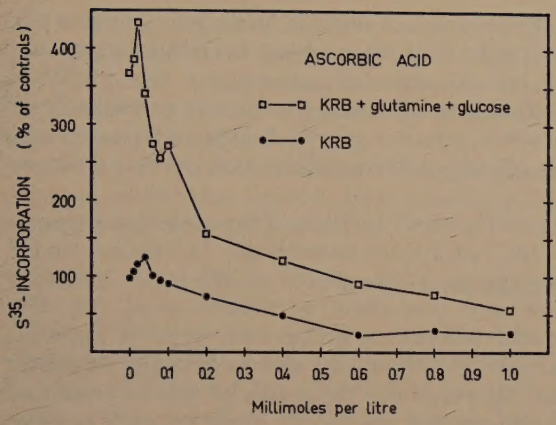

Fig. 6. Experiment D. Inhibitory action of ascorbic acid on the incorporation of S^{35} into CSA *in vitro*. Inhibition amounted to about 80 per cent at 1 mM/l, regardless of whether glutamine (0.1 mM/l) plus glucose (10.0 mM/l) had been added to the KRB or not.

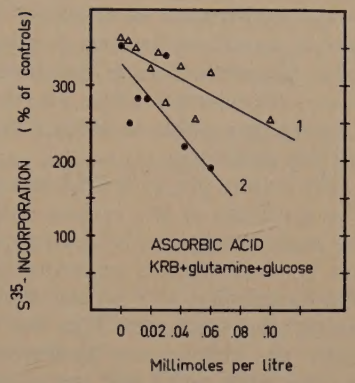

Fig. 7. Experiment D. Ascorbic acid significantly inhibited the incorporation of S^{35} into CSA *in vitro* when added, in concentrations within the normal blood level range, to KRB containing glutamine (0.1 mM/l) and glucose (10.0 mM/l). Regression lines: $y = 355 - 1.1\ x$, $\varrho = 0.77^{**}$ (1); $y = 333 - 2.4\ x$, $\varrho = -0.86^{*}$ (2).

inhibition at these concentrations. The experiment was run in two series, and glutamine plus glucose were added to all flasks.

Similar results were obtained with some analogues of *l*-ascorbic acid, viz., *d*-isoascorbic acid, *l*-gluco-ascorbic acid, and *l*-dehydro-ascorbic acid.

To rule out the possibility that the inhibitory action of ascorbic acid was a mere acid effect, ascorbic acid was added in increasing concentrations to KRB, and the pH measured with a glass electrode. Ascorbic acid had no measurable influence on the pH of the incubating medium (~ 7.4) in concentrations up to 1 millimol per liter.

Discussion

The technique of pair-feeding was introduced by Mitchell (15) in order to compensate for differences in food consumption in nutritional experiments. Although pair-fed guinea-pigs have the same caloric intake as vitamin C-deficient animals, their ultimate weights are higher (1, 14). According to Sheppard and McHenry (24) the divergence is due mainly to variations in water retention. The pair-fed animals have normal ascorbic acid levels in the blood, lack the Göthlin sign (11) and do not show microscopic evidence of scurvy (13).

In the present investigation it was found that the incorporation of S^{35} into rib CSA was reduced in pair-fed as well as scorbutic animals. This reduction occurred *in vivo* after 23 days on the vitamin C-deficient diet. *In vitro* no change was observed at 7–8 and 14–15 days. After 21–22 days, however, a reduction of S^{35} incorporation was measured. These results mainly accord with those reported in vitamin C-deficient animals by Reddi and Norström (19), Kodicek and Loewi (12), Upton and Odell (27)

and Moltke (16). Yet the present results in pair-fed animals bring into question the causal significance of vitamin C in this reduction. In a recent investigation (8) the author found, moreover, a simultaneous reduction of radioactivity due to S^{35} in other tissues too. It is not possible, therefore, to draw any final conclusions from *in vivo* experiments alone. *In vitro*, however, many *in vivo* factors are eliminated and it seems reasonable to ascribe the reduced radioactivity of the cartilage to a decrease in CSA synthesis.

The sulfate group in CSA represents only a small fraction of the disaccharide unit. The synthesis of the carbon chain of the latter must accordingly be studied under the same conditions before any conclusions as to the effects on the entire molecule can be drawn. Such experiments have been performed by Schiller *et al.* (22, 23), who found that the carbon skeleton and the sulfate group turn over at approximately the same rate. This was found both in normal rats and rabbits and in others under the influence of hydrocortisone. However the turnovers of the hexosamine, the hexuronic acid and the sulfate group are not necessarily equal in such a grave condition as scurvy after 3 weeks depletion of vitamin C. In the present investigation the incorporation of C^{14} from uniformly labeled glucose *in vitro* parallelled that of S^{35} both in pair-fed and scorbutic animals. *In vivo*, however, there was an increased incorporation of C^{14}, while that of S^{35} was decreased. The increase in C^{14} activity was highest in the pair-fed animals. These discrepancies may possibly be the result of differences in pool volumes of the various precursors involved. Hence, synthesis of the entire CSA molecule from its three main parts conceivably occurs at the same rate. This might also apply to the present finding that the activities of glucuronic acid were 2–3 times higher than those of hexosamine.

The results obtained by pair-feeding imply that inanition plays a dominant role in reducing the synthesis of rib cartilage CSA. Thus inanition might cause a general growth retardation or an inhibition due to lack of some specific factor(s) other than ascorbic acid but necessary for the normal formation of CSA.

The observations on the recovery animals agree with this concept. They had a normal food consumption and gained weight rapidly. The cartilage of these animals showed *in vivo* a tendency towards normalization of the incorporation of S^{35} into the CSA; *in vitro* this incorporation was normalized.

The *in vitro* experiments (B, D), lastly, show that ascorbic acid does not promote the incorporation of S^{35} into CSA. In higher concentrations the acid was found to have an inhibitory action. Little significance can be attached, however, to this inhibiting effect since it could be produced by some analogues of ascorbic acid as well. Furthermore, a multitude of compounds are known to inhibit the *in vitro* incorporation of S^{35} into CSA (5, 12).

SUMMARY

The incorporation of S^{35} from $Na_2S^{35}O_4$ and C^{14} from uniformly labeled glucose into rib cartilage CSA was studied *in vivo* and *in vitro*. The experiments were conducted on a total number of 231 guinea pigs. The following four categories were examined: (1) vitamin C deficient guinea pigs; (2) pair-fed controls; (3) normal controls; and (4) recovery animals that had been given a vitamin C supplement after three weeks on the deficient ration.

The incorporation of S^{35} into CSA *in vivo* was about 28 per cent of normal in the scorbutic and pair-fed groups; in the recovery group it was 43 per cent. The incorporation of C^{14} was increased, however, in scorbutic and pair-fed animals.

In vitro, the incorporation of S^{35} into CSA was not affected during the first 15 days on the scorbutogenic diet. After 21–22 days the incorporation of S^{35} was about 40 per cent of normal in scorbutic as well as pair-fed animals. The recovery animals showed normal S^{35} incorporation. The incorporation of C^{14} was about 47 per cent of normal in scorbutic animals and about 62 per cent of normal in pair-fed controls.

Ascorbic acid did not increase the S^{35} incorporation into CSA from scorbutic guinea pigs *in vitro*.

It is concluded that inanition plays a dominant role in the reduced synthesis of CSA in the advanced stage of acute scurvy.

ACKNOWLEDGMENTS

This investigation was aided by grants from *Konung Gustaf V:s 80-årsfond, Stiftelsen Thérèse och Johan Anderssons Minne, Reservationsanslaget* of Karolinska Institutet, and by a grant to Professor G. Häggqvist from *Stiftelsen Gustaf och Tyra Svenssons Minne.*

The author is indebted to Miss L. Berkow, Mrs. M. Björkman, Miss K. Carlsson, Mrs. R. Flagell and Mr. H. Johnsson for skilled technical assistance. The flow-counter was kindly placed at my disposal by Professor L. Friberg. Dr. V. Hall constructively criticized the manuscipt. The English manuscript was revised by Mr. Stanley Vernon. ASTRA, Inc., Södertälje, Sweden, generously supplied the vitamins necessary for the experiments.

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Tryckt den 22 april 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 44

Communicated 9 April 1958 by Arne Westgren and Gunnar Hägg

An X-ray investigation of the structure of silver iodide, silver thiocyanate, and silver thiosulphate complexes in solution

By Rolf O. Nilsson

With 12 figures in the text

The structure of molecules containing heavy atoms dissolved in a solvent containing light atoms has in some cases been determined by X-ray scattering. Brosset [1] proved the presence of $Mo_6Cl_8^{4+}$ in an alcoholic solution of $[Mo_6Cl_8]Cl_6 2H_3O \cdot 6H_2O$ and Vaughan, Sturdivant, and Pauling [2] determined the structures of platinum, niobium and tantalum complexes in organic solvents. It is also possible to determine the structure of molecules containing the same kind of atoms as the solvent. Thus Grjotheim and Krogh-Moe [3] successfully studied the structure of sulphur molecules dissolved in carbon disulphide.

The aim of the present investigation was to determine the structure of some silver complexes in water solutions. Lithium iodide, ammonium thiocyanate, and sodium thiosulphate solutions saturated with a corresponding silver salt were investigated.

Theory

In this paper the electron distribution function introduced by Finbak [4] will be used. The pair distribution function $\sigma_{(r)}$ (the probability of finding an electron at the distance r in a spherical shell of thickness $\mathrm{d}r$) is defined

$$\sigma_{(r)} = \int_V \int_f \varrho_{(\mathfrak{p})} \varrho_{(\mathfrak{p}+r)} \, \mathrm{d}f \, \mathrm{d}V, \tag{1}$$

where $\varrho_{(\mathfrak{p})}$ is the electron density at the point p, $\int_V \mathrm{d}V$ is the integration over the whole volume V of the sample and $\int_f \mathrm{d}f$ is an integration over the surface of a spherical shell of radius r with the center at p. The total coherent X-ray intensity scattered from the sample is then:

$$I_{t(s)} = \int_0^\infty \sigma_{(r)} \frac{\sin sr}{sr} \, \mathrm{d}r. \tag{2}$$

s is defined as $(4\pi \sin \theta)/\lambda$. 2θ is the angle between the incident and the scattered beam and λ is the wavelength of the radiation.

The scattering from electrons within the same atom is given by

$$N f_{m(s)}^2 = \int_0^\infty \sigma_{at(r)} \frac{\sin sr}{sr} \, dr \tag{3}$$

and the scattering at zero and very small angles by

$$I_{0(s)} = \int_0^\infty 4\pi r^2 \varrho_0^2 V \frac{\sin sr}{sr} \, dr. \tag{4}$$

The relation between these quantities is (cf. Krogh-Moe [5])

$$I_{t(s)} - I_{0(s)} - N f_{m(s)}^2 = \alpha N I_{e(s)} - N (f_{m(s)}^2 + I_{i(s)})$$

$$= N I_{(s)} = \int_0^\infty [\sigma_{(r)} - \sigma_{at(r)} - 4\pi r^2 \varrho_0^2 V] \frac{\sin sr}{sr} \, dr, \tag{5}$$

where N is the total number of atoms, f_m is the scattering factor of atom m, αN is a factor which brings the observed intensity (corrected for polarization and absorption) to an absolute scale, $N I_{i(s)}$ is the incoherent part of the scattered intensity and ϱ_0 is the mean electron density of the sample with volume V. To obtain $N I_{(s)}$ the sum of the incoherent scattering and the intensity scattered by electrons within the same atom is subtracted from the total scattered intensity. This sum, which can be calculated from literature data, is called the background scattering. Atomic scattering factors have been calculated by Viervoll and Ögrim [6] up to $s = 30$. The incoherent scattering is given by $(Z - \Sigma f_i^2)/B^3$. Z is the number of electrons in the atom, f_i is the scattering factor of the i'th electron (Σf_i^2 is given by Compton and Allison [7]) and B is the Breit–Dirac recoil correction factor. The incoherent scattering of atoms for which Σf_i^2 is not tabulated was calculated according to Bewilogua [8].

A Fourier transform applied to equation (5) gives

$$\sigma'_{(r)} = \sigma_{(r)} - \sigma_{(at)} - 4\pi r^2 \varrho_0^2 V = \frac{2r N}{\pi} \int_0^\infty s \, I_{(s)} \sin sr \, ds. \tag{6}$$

This integral was in the numerical calculations approximated by

$$\sum_0^{s_0} s \, I_{(s)} \sin sr \, \Delta s. \tag{7}$$

The sum was calculated with $\Delta s = 0.2$ (in the case of solution 1 in the iodide system also for $\Delta s = 0.1$, which gave approximately the same result). s_0 was taken to 9.9 in most cases. The calculations were partly performed on a hand calculator and partly with a punch card machine.

The positions of the maxima in the radial distribution function $\sigma'_{(r)}$ give the distances between the atoms in the solution.

Method

When this investigation was started no counter equipment was available and the scattering was therefore registered on photographic films. The sample was enclosed in a glass capillary and mounted in the center of a Weissenberg camera rebuilt for liquid scattering work. The capillary was rotated in the evacuated camera ($p \approx 5$ mm Hg) during the exposure. A parallel X-ray beam (Mo $K\alpha$), monochromated by reflection from the *200* plane of a LiF crystal was used. The exposure time was about 6 hours. An empty capillary gave no noticeable blackening on the film in that time. When the sample had been irradiated, a millimeter scale and a logarithmic blackening scale were exposed on a not irradiated part of the same film. The millimeter scale was applied to avoid errors due to shrinkage of the film. The blackening was recorded photometrically. The logarithmic scale made it possible to read off the scattered intensity in the nonlinear range of film blackening. The recorded intensities were corrected for polarization by dividing with a polarization factor calculated according to equation (8a).

$$P = \frac{1 + 0.8787 \cos^2 2\theta}{2} \quad \text{(for Mo } K\alpha\text{)} \tag{8a}$$

The intensities were also corrected for absorption by dividing with absorption factors, A, calculated for cylindrical samples bathed by X-rays. 100 A is tabulated as a function of the scattering angle for some values of μr_c in International Tables [9]. Here r_c is the inner radius of the capillary and μ, the absorption, is given by

$$\mu = \varrho_1 \sum \frac{\mu_m}{\varrho} \tag{8b}$$

where ϱ_1 is the density of the sample and μ_m/ϱ is the mass absorption coefficient of atom m. The intensities, corrected for polarization and absorption, were multiplied by a constant so chosen that the intensity curve coincided with the background curve at high values of s. Fig. 1 shows that the intensity curves so corrected (dashed curves) lie under the background curves and that the deviation increases for rising values of μr_c (cf. Tables 1–3). The absorption correction can be avoided by measuring the *reflection* from the surface of a sample, keeping the incident angle equal to the angle between the surface and the reflected beam. Fortunately a diffractometer with counter equipment in which this condition could be fulfilled was at disposal at a later stage of this investigation. With this diffractometer solutions number 1 in the iodide, the thiocyanate, and the thiosulphate systems were reinvestigated. The solutions were kept in a cell made by a milled out piece of polystyrene. The cell was covered with an aluminium foil which had been oxidized to avoid corrosion by the solutions. The diffractometer equipment was not evacuated during the measurements. A parallel beam of Mo radiation was used. Monochromatization was achieved with a pair of balanced zirconium strontium filters placed between the X-ray tube and the sample. Five to fifteen thousand impulses were registered in each point of measurement. About seventy per cent of the intensity registered with the zirconium filter was registered with the strontium filter and the error in the difference between the intensities transmitted by the two filters is consequently rather high. After correction for polarization the curves were adapted to their

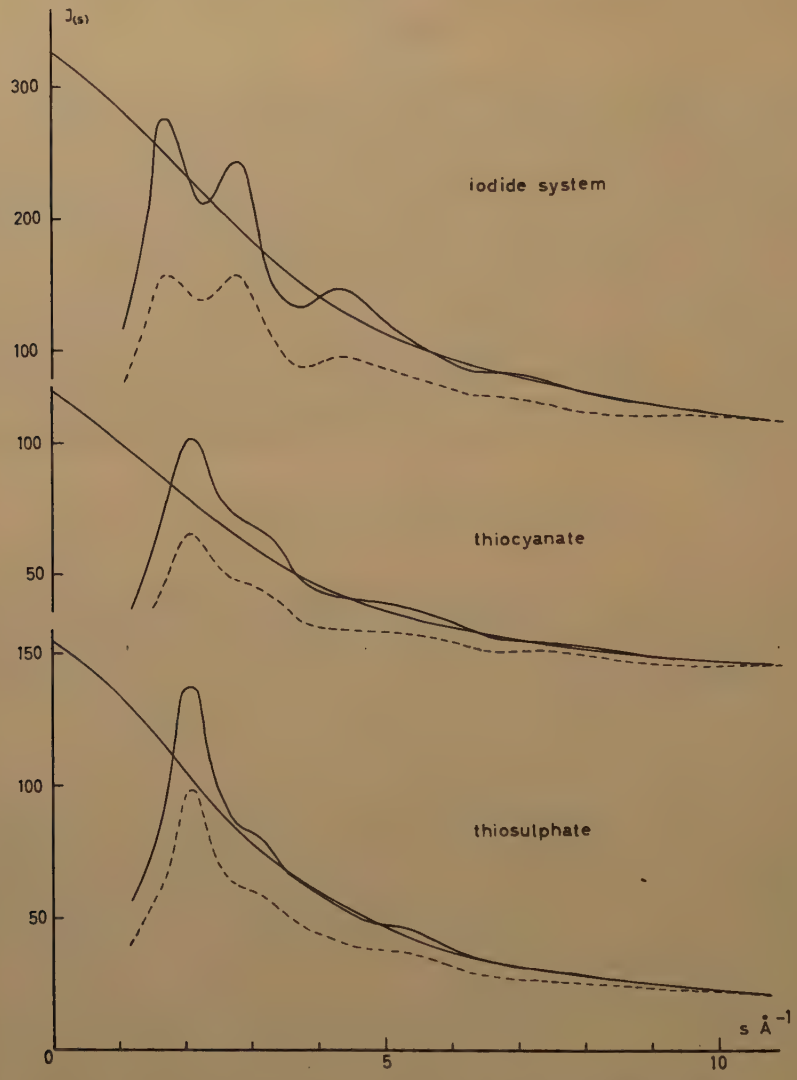

Fig. 1. Intensity curves for the solutions number 1 in the three investigated systems. The dashed curves which are obtained by the photographic method are corrected for absorption according to Ref. [9]. The full drawn curves are obtained by reflection from flat specimens. The background curves are calculated for the same number of silver atoms in all three systems.

respective background curves by a method proposed by Norman [10] and independently by Krogh-Moe [5]. Equation (18) in Ref. [5] is

$$-2\pi^2\varrho_0^2\frac{V}{N}=\alpha\int_0^{s_0}s^2 I_{e(s)}\,\mathrm{d}s-\int_0^{s_0}(f_{(s)}^2+I_{1(s)})s^2\,\mathrm{d}s. \qquad (9)$$

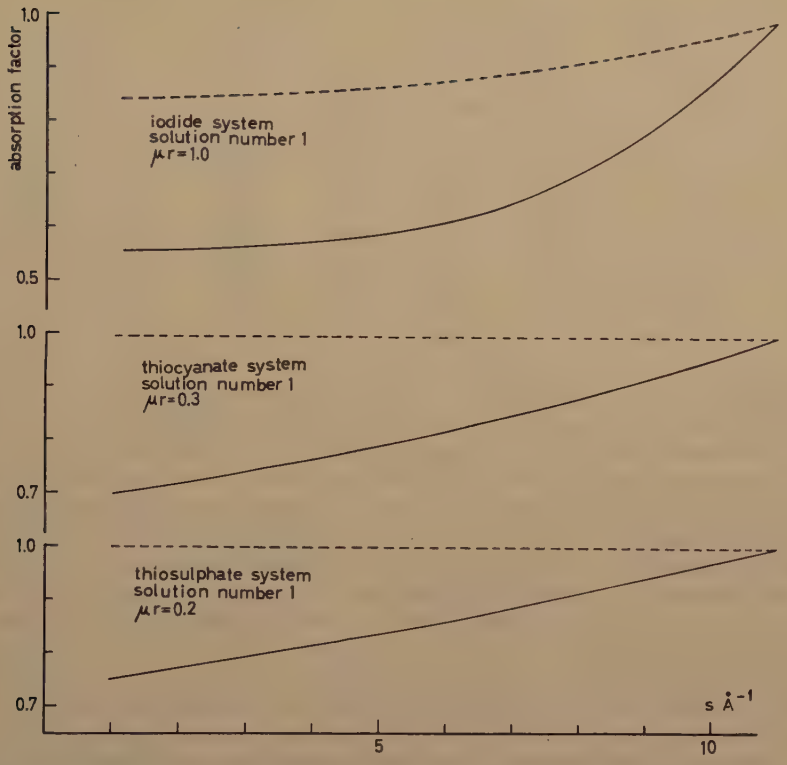

Fig. 2. Empirical absorption factors (full-drawn curves) obtained from Fig. 1 compared with absorption factors (broken curves) calculated according to Ref. [9] for cylindrical samples.

The two integrals were obtained graphically (the upper limit $s_0 = 9.9$ was used) and as the left-hand side could be calculated from the densities of the samples α was easily obtained. The observed intensities were then multiplied with αN and plotted against s. The curves obtained in this way (full-drawn in Fig. 1) oscillate around the background curves in contrast to the dashed ones. In Fig. 2 absorption factors evaluated according to Ref. [9] are compared with empirical absorption factors obtained from the deviations in Fig. 1. The calculated absorption factors are obviously not valid for the silver solutions investigated here. Consequently absorption corrections of the same form as the empirical ones in Fig. 2 were used to correct the observed scattered intensities from the cylindrical samples.

The iodide system

It is known that iodide solutions can dissolve appreciable amounts of silver iodide (concentrated HI solution saturated with AgI contains 5.2 M AgI and 8.1 M HI, Erber [11]) under formation of anionic complexes. This complex formation has been studied by solubility measurements (Leden [12]) and potentiometric measurements (Leden [13]) at 25°C and the ionic strength 4 M. Leden assumes that mononuclear

Table 1. Solutions investigated in the iodide system.

Solution number	c_{Ag} mmol/g	c_I mmol/g	c_{H_2O} mmol/g	ϱ_l g/ml	μr_c	r_c cm
1	1.54	4.41	14.2	2.421	1.04	0.016
2	1.41	4.20	16.5	2.223	0.86	0.015
3	1.26	3.96	18.9	2.088	0.75	0.015
4	0.875	3.38	25.4	1.726	0.67	0.019
5	0.659	3.09	28.9	1.588	0.43	0.015
6	0.720	4.54	17.8	2.076	1.14	0.022
7	0	2.89	34.2	1.391	0.32	0.015

groups AgI, AgI_2^-, AgI_3^{2-}, and AgI_4^{3-} as well as polynuclear ions of the form $Ag_mI_{2m+2}^{(m+2)-}$ are formed in solutions containing excess iodide ions.

In the present investigation the structure of silver iodide complexes in lithium iodide solutions saturated with silver iodide was studied. Lithium iodide was used instead of sodium iodide because of its lower scattering effect. The silver concentrations lie in the range $0.66 < c_{Ag} < 1.54$ mmol/g. Table 1 gives the composition of the solutions as determined by analysis. Fig. 3 shows the intensity functions (obtained in the way described in the preceding section) of some of the samples. The solution with the highest silver concentration gives a characteristic scattering pattern. The radial distribution function is calculated according to (7). The $\sigma'_{(r)}$ curves obtained in this way (dashed curves in Fig. 4) represent a distribution of all the interatomic distances in the solution. To obtain the electron distribution within the complex ions a distribution function arising from the "solvent" was subtracted from the original $\sigma'_{(r)}$ function. The resulting distribution curves (full-drawn in Fig. 4) however still contain a contribution from the distances between the complex ions and the "solvent" molecules and distances between the complex molecules. The curves in Fig. 4 have been calculated for the same number of silver atoms, 0.047. The "solvent" in the investigated solutions was water, free iodide ions, and lithium ions. It is not possible to prepare a lithium iodide solution which is identical with the "solvent" in the silver solutions since in these solutions there are about three times as many lithium ions as free iodide ions. As an approximation a $4M$ lithium iodide solution was investigated. The ratio of iodide ions to water in this solution is about the same as the ratio free iodide ions to water in the complex solutions. The distribution functions of the lithium iodide solution which were subtracted from the $\sigma'_{(r)}$ for the silver solutions were calculated for the number of iodide ions which corresponded to the number of estimated free iodide ions in the silver solutions. The lowest curve in Fig. 4 is calculated for 0.042 iodine atoms.

The distribution function of solution number 7 shows a maximum at ≈ 3.8 Å which approximately corresponds to the sum of the radii of oxygen and iodine (3.7 Å). The hydration of the iodide ions thus obviously disrupts the structure of pure water, which shows a peak in the distribution function at $r = 2.8$ Å and a minimum at 3.5 Å (Morgan and Warren [14]). A similar disruption with a maximum at 3.2 Å has been found in 25 % KCl solution by Brady and Krause [15]. The peak at 3.8 Å in the distribution function of the lithium iodide solution also appears in the dashed curve belonging to solution number 5 in Fig. 4 and especially in the curve belonging to solution number 6 which has a high ratio of iodine to silver.

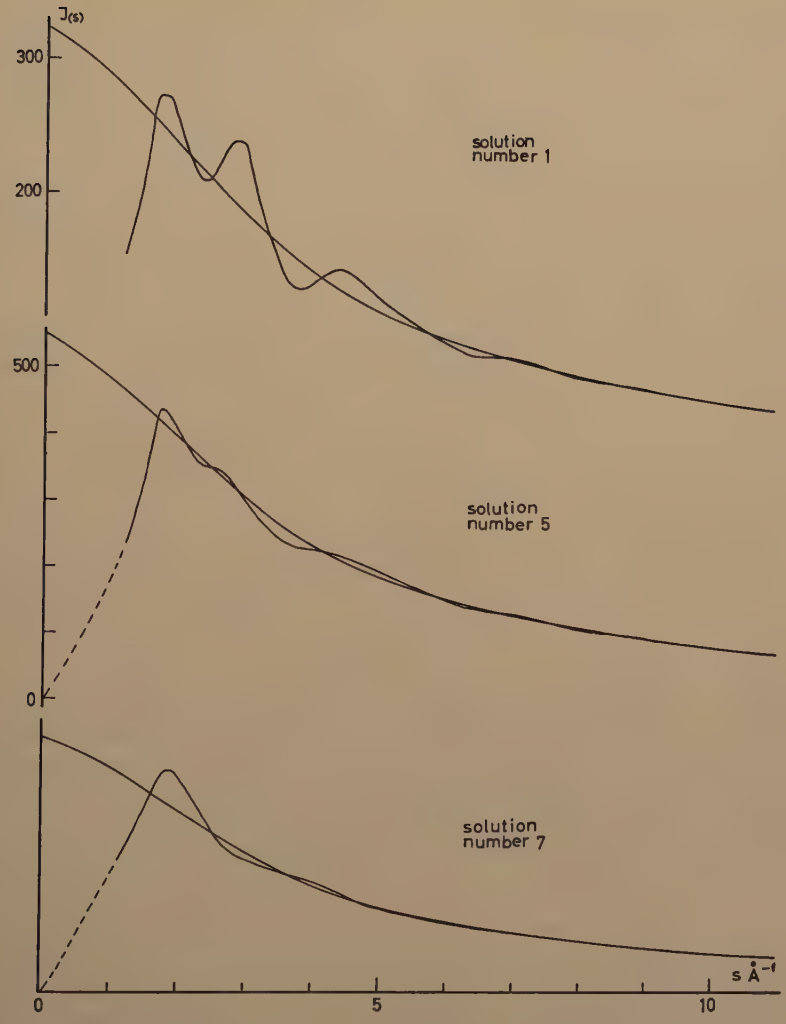

Fig. 3. Some of the intensity curves in the iodide system corrected for absorption by empirical absorption factors. The background curves for the solutions numbers 1 and 5 are calculated for the same number of silver atoms.

The distribution functions of solutions 1–5 will now be discussed. Some of the full-drawn curves, which represent $\sigma'_{(r)\,\text{solution}} - \sigma'_{(r)\,\text{"solvent"}}$, in the interval $r < 2$Å show small peaks which have no physical meaning. The first main peak, which has the same height for solutions 1–5, occurs at 2.9 Å. The sum of the covalent radii of silver and iodine is 2.86 Å and consequently the maximum at 2.9 Å most probably corresponds to the silver–iodine distance. The next main peak (which has the same height for solutions 1–5) occurs at 4.7 Å which is the length of an edge in a tetrahedron in which the distance from center to corner is 2.9 Å. This consequently shows that the silver atoms are tetrahedrally surrounded by iodine atoms. (From the curves

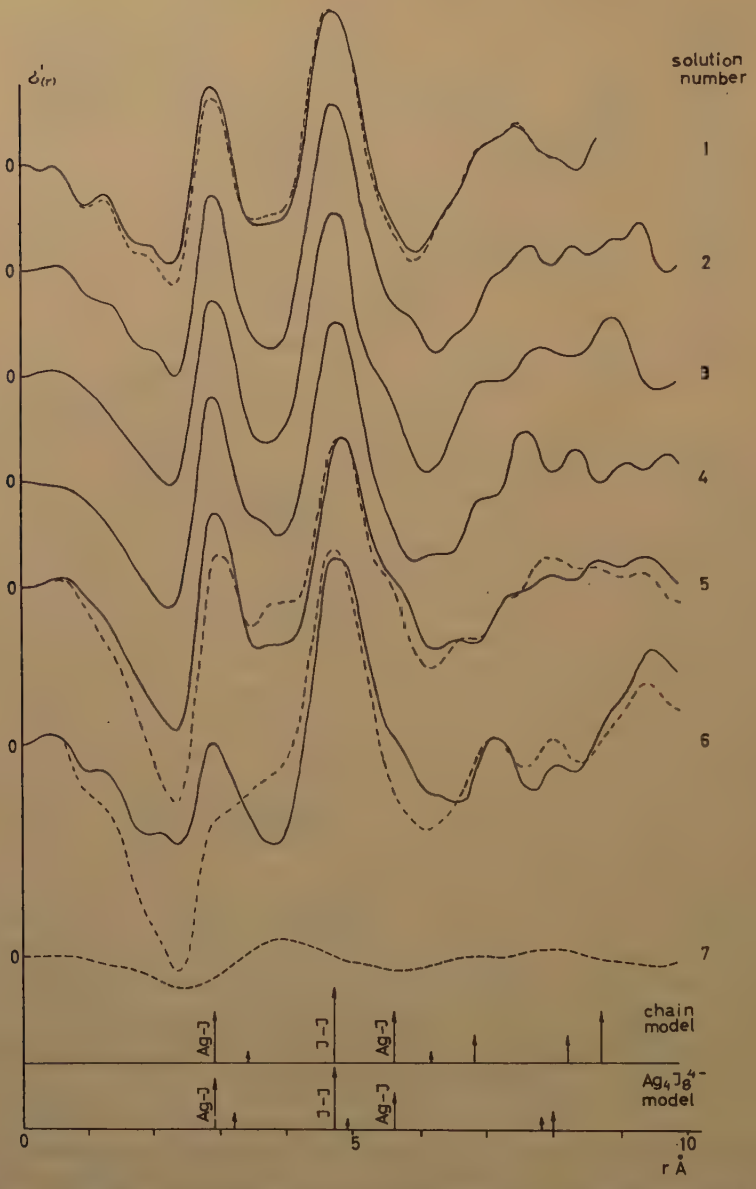

Fig. 4. Electron distribution functions in the iodide system. The curves belonging to solutions numbers 1 to 6 have been calculated for the same number of silver atoms (0.047) while the curve belonging to solution number 7 is calculated for 0.042 iodine atoms. The dashed curves represent $\sigma'_{(r)}$ for the investigated solutions and the full-drawn ones give $\sigma'_{(r)\text{ solution}} - \sigma'_{(r)\text{ "solvent"}}$.

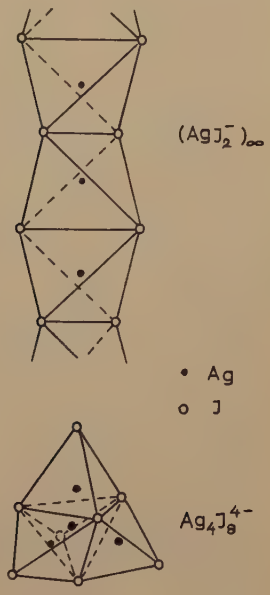

Fig. 5. Structure models proposed for the polynuclear silver iodide complexes.

in Fig. 4 it is not possible to say if the silver atoms are tetrahedrally surrounded by iodine atoms or *vice versa* as the atomic numbers of silver and iodine are of the same order of magnitude. Other measurements (Refs. [12] and [13]) show, however, that the complexes are richer in iodine than in silver and therefore silver must be the central ion.) In a later section it will be shown that all four corners are occupied by iodine atoms. The peak at 4.7 Å is not symmetrical, probably as a consequence of a superimposed peak at approximately 5.4 Å. The distribution functions at r larger than 6 Å show high ripples and conclusions drawn from this part of the curve must be regarded as less reliable.

Solution number six which has a rather high iodine content and a rather low silver content was prepared in order to shift the equilibrium towards mononuclear complexes. The dashed curve shows no minimum between 2.9 and 4.7 Å in contrast to the full-drawn curve due to the great influence of the "solvent". The peak at 4.7 Å on the resulting curve is higher and the peak at 2.9 Å is lower than the corresponding maxima on curves 1–5. This change in the heights of the peaks is in accordance with a shift towards mononuclear groups but as the method is afflicted with considerable errors it is not possible to draw definite conclusions from this effect.

It was also tried to determine the coordination number of the silver atom in solution number 1 by analyzing the function

$$\sigma_{(r)} - \sigma_{(at)} = \frac{2rN}{\pi} \int s I_{(s)} \sin sr \, \mathrm{d}s + 4\pi\varrho_0^2 V r^2. \qquad (10)$$

$\sigma_{(r)} - \sigma_{(at)}$ was drawn against r in Fig. 6 and then theoretical peaks were calculated according to the following expression (cf. Viervoll [16]) and compared with the experimental curve.

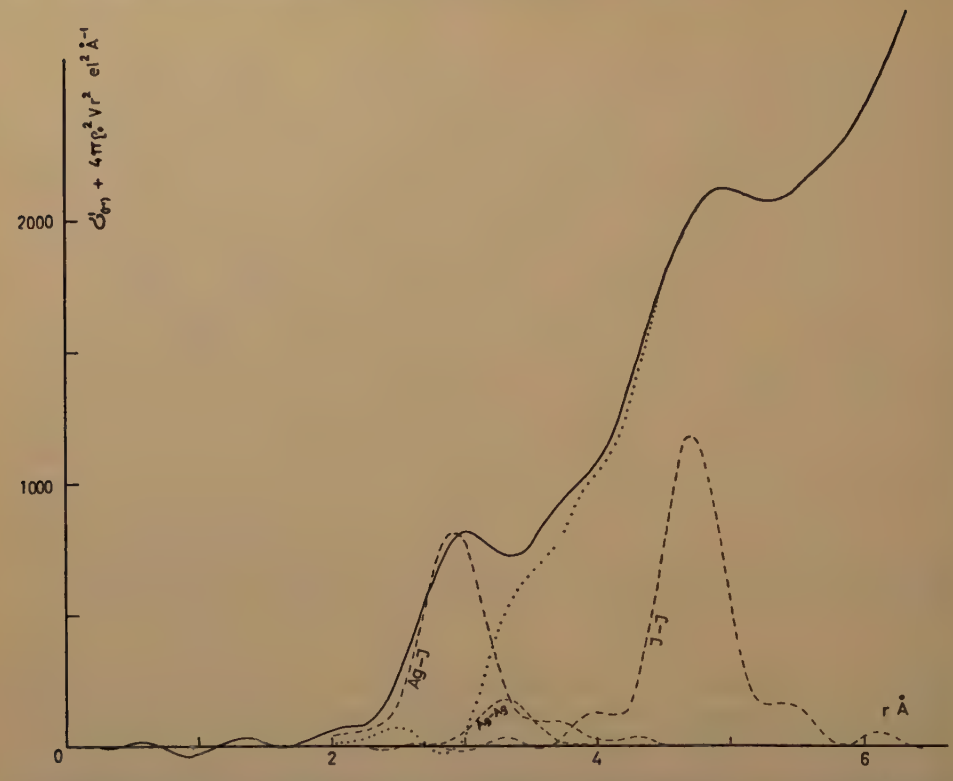

Fig. 6. Evaluation of the coordination number of silver with respect to iodine in solution number 1. The dotted curve obtained after subtraction of the Ag–I peak, calculated with the coordination number of silver equal to four.

$$\frac{2 n_{m,n} N 2r}{R} \int f_m f_n \sin sR \sin sr \, ds. \qquad (11)$$

The peak calculated for the Ag–I distance $R = 2.9$ Å coincided rather well with the experimental one for $n_{m,n} = 4$ ($n_{m,n}$ is here the coordination number of silver with respect to iodine). A still better agreement would have been found if a temperature factor had been used in the calculations. Peaks calculated for a I–I distance ($R = 4.7$, $n_{m,n} = 5$) and a Ag–Ag distance ($R = 3.3$, $n_{m,n} = 2$) are also shown in Fig. 6. These coordination numbers occur in a chain built up from tetrahedra sharing edges (cf. Fig. 5). A study of the volume requirements in the solution shows that the area under the curve between 3 and 4.7 Å can be approximately explained with I–O (the main part) and I_{free}–$I_{complex}$ distances. As the peaks at larger distances than 3 Å do not contribute in any appreciable degree to the area under the peak at 2.9 Å the coordination number 4 of silver may be regarded as established.

A similar analysis was made for the most diluted solution (number 5). In this case it was reasonable to subtract from the distribution curve for the whole solution a radial distribution curve arising from the "solvent". The composition of the solution is nearly the same as one of the solutions investigated by solubility measure-

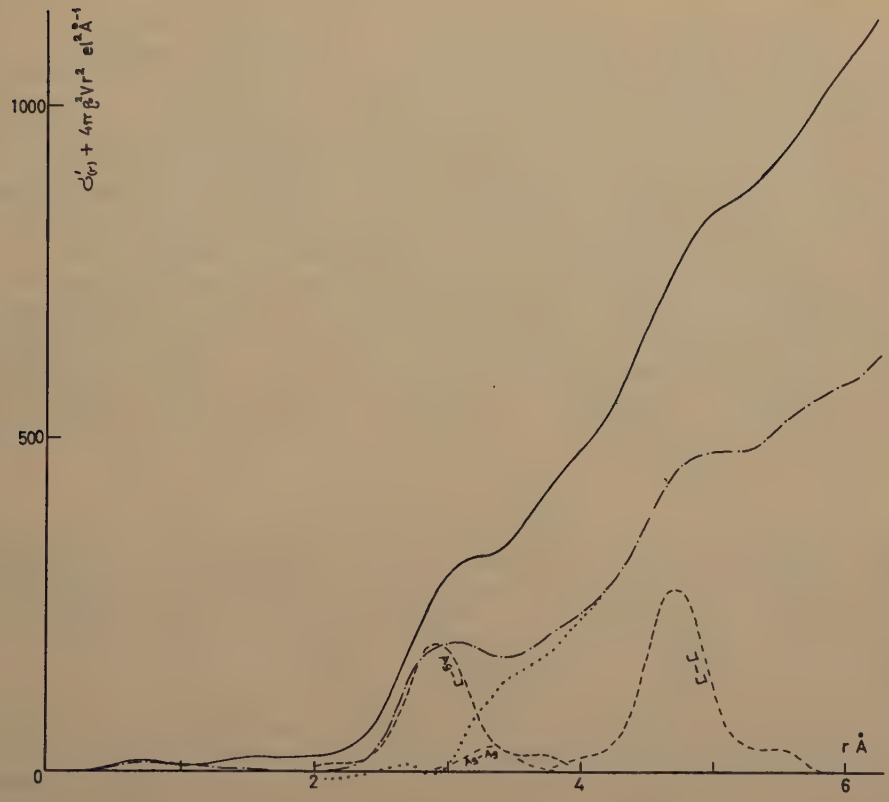

Fig. 7. Evaluation of the coordination number of silver with respect to iodine in solution number 5. ———, the ordinate function for the total solution; — · — · —, the ordinate function after subtraction of the "solvent"; · · ·, after subtraction of the Ag–J peak, calculated with coordination number four.

ments (lithium ions are used instead of sodium ions, however) and the free iodide concentration could be taken from Ref. [12]. The result is shown in Fig. 7 where the Ag–I peak calculated with the coordination number 4 is seen to coincide with the experimental one.

From Figures 4, 6, and 7 the conclusion was drawn that the silver atom is tetrahedrally surrounded by four iodine atoms at the distance 2.9 Å. As the ratio of iodine to silver is less than four in the solutions number 1–4 the X-ray measurements consequently implicate that these solutions contain polynuclear species. The polynuclear ions according to Leden have the composition $Ag_mI_{2\,m+2}^{(m+2)-}$ but he also claims that this series may be changed to $Ag_mI_{2\,m}^{m-}$ at some high value of m. Two models (Fig. 5) were used to explain the curves in Fig. 4. One of them is a chain built up by tetrahedra sharing edges and the other is a symmetrical ion $Ag_4I_8^{4-}$ built up from four silver iodine tetrahedra, each sharing three edges with the others. The positions of the arrows in Fig. 4 coincide rather well with the peaks in the $\sigma'_{(r)}$ functions. To get an idea of the magnitude of a peak arising from a certain pair of atoms, the arrows have been calculated as the product of the

atomic numbers of the two atoms in question and the frequency with which the distance occurs in the molecule. The two models seem to fit the experimental curves with approximately the same accuracy and it is not possible to say if the ions consist of chains or of separate groups. It should have been interesting to know the structure of a crystal of the composition $MAgI_2$ (M = alkali metal) but unfortunately no such structure is known. The chain model is supported, however, by the fact that crystalline $CsAg_2I_3$ (Brink, Binnendijk, and van de Linde [17]) contains tetrahedra linked up to double chains by sharing edges. Crystalline K_2AgI_3 (Brink and Kroese [18]) consists of single chains built up by tetrahedra sharing corners.

The structure of silver iodide species in water–acetone mixtures has been investigated by Dallinga and Mackor [19] who studied one solution which should contain mainly AgI_2^- ions (with silver in the center of an aceton–iodine tetrahedron) and one which should contain $Ag_4I_6^{2-}$ ions (the structure of which is regarded as a slightly deformed part of the α-AgJ lattice). One may ask, however, if not the distribution function which they obtained from the polynuclear solution could be explained by other structure models as well.

The thiocyanate system

The complex formation between silver and thiocyanate ions has been studied by Leden and Nilsson [20] by solubility measurements and potentiometric measurements at 25°C. The solutions had the ionic strength $4\,M$ with sodium perchlorate as additional salt. The existence of AgSCN, $Ag(SCN)_2^-$, $Ag(SCN)_3^{2-}$, $Ag(SCN)_4^{3-}$, and polynuclear ions of the form $Ag_m(SCN)_{2\,m+2}^{(m+2)-}$ was proved.

In the present work ammonium thiocyanate solutions saturated with silver thiocyanate were investigated. Ammonium thiocyanate was used instead of sodium thiocyanate as the solubility of silver thiocyanate is greater in ammonium thiocyanate [21]. Table 2 gives the composition of the investigated solutions. The observed intensities were corrected as described in an earlier section. Fig. 8 shows the $I_{(s)}$ curves for some of the solutions and Fig. 9 gives the distribution functions. From $\sigma'_{(r)}$ (dashed curves) was subtracted a distribution function arising from the "solvent". From the resulting $\sigma'_{(r)\,\text{solution}} - \sigma'_{(r)\,\text{"solvent"}}$ curves (full-drawn in Fig. 9) the following conclusions can be drawn. The ripples for $r < 2$ Å are not reproducible and have no physical meaning. In the thiocyanate ions the distances S–C ≈ 1.7 Å and C–N ≈ 1.1 Å occur but as these atoms are light they give a negligible contribution to the distribution functions at 1.7 and 1.1 Å. The peak at $r = 2.6_5$ Å arises from the silver–sulphur distance. The distance Ag–S has been determined in crystalline silver thiocyanate (Lindqvist [22]) to 2.43 Å in the –Ag–SCN–Ag–

Table 2. Solutions investigated in the thiocyanate system.

Solution number	c_{Ag} mmol/g	c_{SCN} mmol/g	c_{H_2O} mmol/g	ϱ_l g/ml	μr_c	r_c cm	Solid phase[a]
1	1.97	5.67	21.7	1.422	0.29	0.024	AgSCN
2	1.65	4.96	26.3	1.338	0.20	0.020	AgSCN
3	1.25	7.67	16.8	1.343	0.19	0.020	$(NH_4)_5Ag(SCN)_6$
4	0	3.42	43.5	1.060	0.05	0.020	

[a] Cf. Ref. [21].

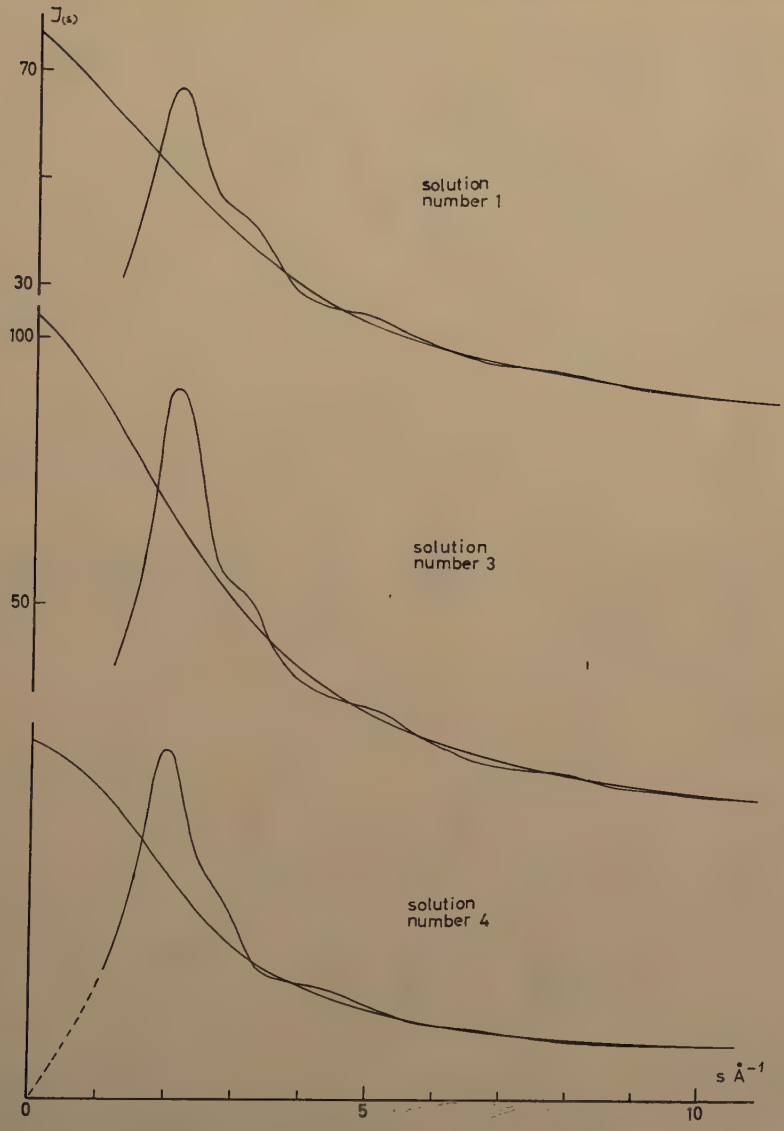

Fig. 8. Intensity functions in the thiocyanate system corrected for absorption by empirical absorption factors.

SCN– chains, which are present in this compound, and to 2.89 and 3.00 Å between the silver atom in one chain and a sulphur atom in the next. The crystal structure of $NH_4Ag(SCN)_2$ has been determined by Lindqvist and Strandberg [23] who found that the silver atom coordinates four sulphur atoms in a very distorted tetrahedron. The structure is according to these authors best described as built up from AgSCN molecules, with Ag–S 2.47 Å, and SCN^- and NH_4^+ ions. The distances from the silver

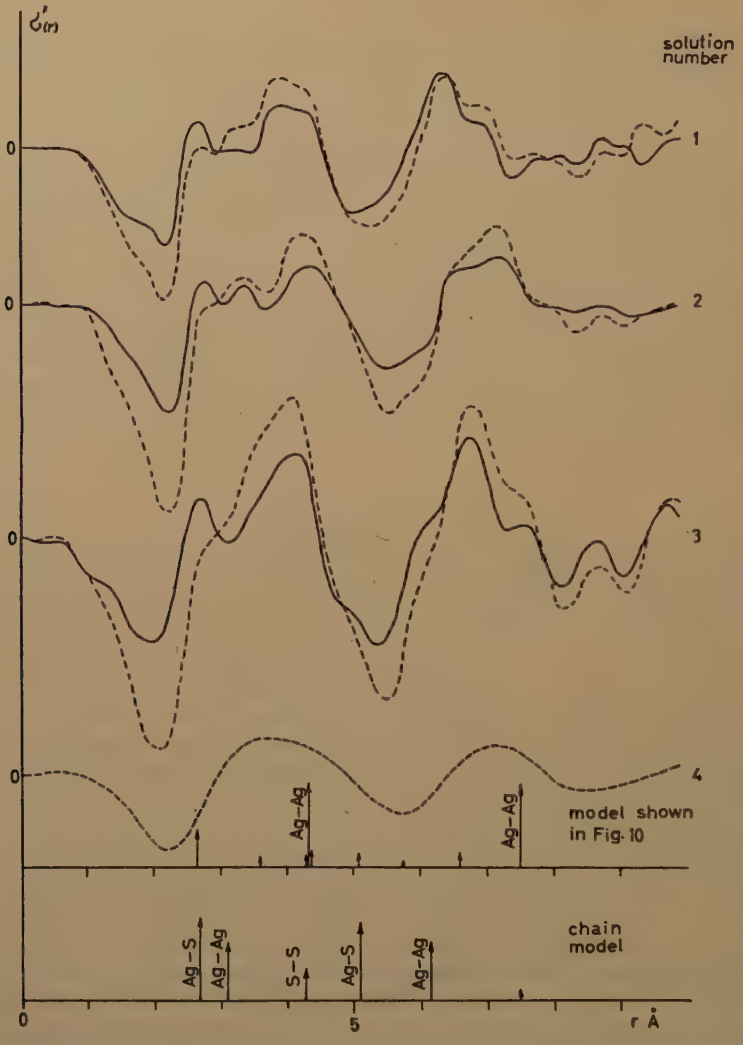

Fig. 9. Electron distribution functions in the thiocyanate system. The curves belonging to solutions numbers 1–3 have been calculated for the same number of silver atoms. The dashed lines represent $\sigma'_{(r)}$ for the investigated solutions while the full-drawn ones give $\sigma'_{(r)\,\text{solution}} - \sigma'_{(r)\,\text{"solvent"}}$.

atom to the sulphur atoms in the thiocyanate ions are 2.63 Å, 2.65 Å, and 2.74 Å. The peak at 2.65 Å in Fig. 9 in this paper is an average of the silver–sulphur distances in the complex ions and does not exclude the possibility that one silver–sulphur distance is shorter than the others as in the crystal $NH_4Ag(SCN)_2$ even if this is less probable. The concentration of *free* AgSCN molecules, in which the distance silver–sulphur probably is 2.45 Å, is less than $10^{-8}M$ (see Ref. [20]). It was also tried to determine the coordination number of silver by the method used in the iodide system.

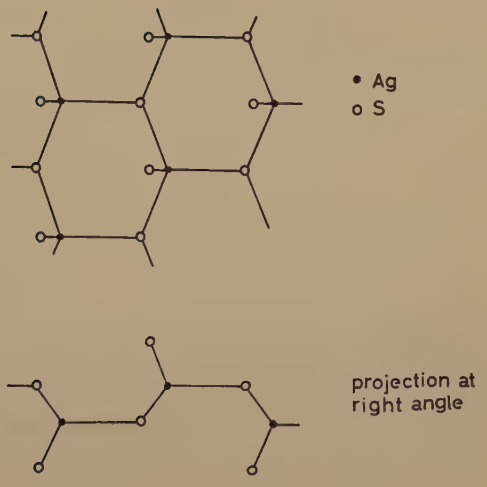

Fig. 10. Structure model with the composition $(Ag(SCN)_2)_\infty$. Carbon and nitrogen atoms omitted.

The $\sigma_{(r)} - \sigma_{(at)}$ function in the thiocyanate system does not, however, give a resolved peak and definite conclusions could therefore not be drawn. The $\sigma'_{(r)}$ curves belonging to solutions 1 and 2 have small peaks at $r \approx 3.3$, ≈ 3.9, and ≈ 4.3 Å. The last-mentioned distance corresponds to the length of an edge in a tetrahedron with the distance from center to corner equal to 2.65 Å. In Fig. 9 arrows have been calculated for two hypothetical models. The first one is a chain model similar to the one shown in Fig. 5 (iodine is replaced by sulphur) which explains the formation of the complexes in the series $Ag_m(SCN)_{2m+2}^{(m+2)-}$, found by the potentiometric measurements. The distances occurring in this model agree rather well with the experimental curve except at 5.1 Å where we have a high arrow while the distribution functions show a minimum. Further the angle Ag–S–Ag is small in this model and it therefore seems to be less probable. The other model represented by arrows in Fig. 9 is shown in Fig. 10. It is an idealized layer structure in which both the silver and the sulphur atoms show tetrahedral angles. It was choiced since the crystal structure of $NH_4Ag(SCN)_2$ can be regarded as a layer structure, the layers containing condensed hexagons with three silver and three sulphur atoms in each ring and in addition one thiocyanate group attached to each silver atom. The model in Fig. 10 gives a silver–sulphur distance equal to 2.65 Å and a silver–silver and a sulphur–sulphur distance of 4.33 Å in agreement with the experimental curve. In the range $2.7 < r < 4.3$ Å the agreement is not so good, however.

The distribution function of solution number 4 does not show the peaks found in pure water but has a flat maximum at 3.7 Å which arises from the distance between water molecules and thiocyanate ions.

The thiosulphate system

The complex formation between silver ions and thiosulphate ions has been studied in 4 *M* $NaClO_4$ at 25°C by solubility measurements (Nilsson [24], other authors in the field have been cited in this reference) and potentiometric measurements (Nilsson [25]). The existence of the ions $AgS_2O_3^-$, $Ag(S_2O_3)_2^{3-}$, $Ag(S_2O_3)_3^{5-}$, and $Ag_m(S_2O_3)_{m+2}^{(m+4)-}$

Table 3. Solutions investigated in the thiosulphate system.

Solution number	c_{Ag} mmol/g	$c_{S_2O_3}$ mmol/g	c_{H_2O} mmol/g	ϱ_l g/ml	μr_c	r_c cm	Solid phase[a]
1	1.30	2.36	23.63	1.553	0.21	0.020	$NaAgS_2O_3 \cdot H_2O$
2	0.764	2.80	27.30	1.6	0.10	0.018	$Na_3Ag(S_2O_3)_2 \cdot 2H_2O$
3	—	1.09	45.9	1.138	0.05	0.020	

[a] Cf. Basset and Lemon [26].

was proved. Of these $Ag(S_2O_3)_2^{3-}$ and $Ag_6(S_2O_3)_8^{10-}$ seemed to be more stable than the others.

The compositions of the solutions investigated by X-ray scattering are given in Table 3. Fig. 11 shows the intensity functions and Fig. 12 the distribution functions (dashed lines) of the investigated solutions. The full-drawn curves in Fig. 12 give the difference between the distribution function of the solution and of the "solvent", *i.e.* a sodium thiosulphate solution with a concentration corresponding to the estimated free thiosulphate concentration in the solution. The two upper curves have a small superimposed peak at 1.5 Å, which arises from the S–O distance within the

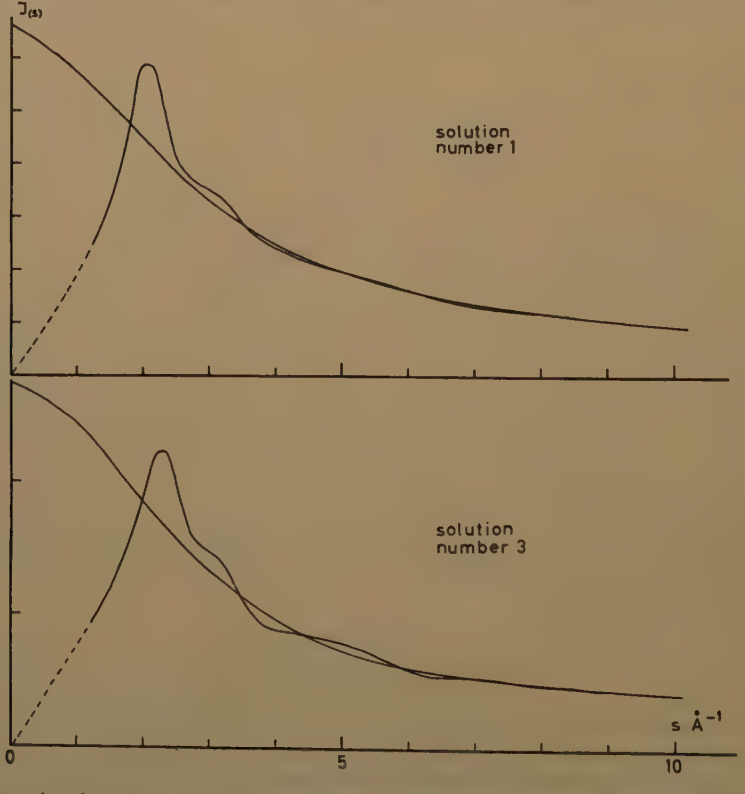

Fig. 11. Intensity functions in the thiosulphate system corrected for absorption by empirical absorption factors.

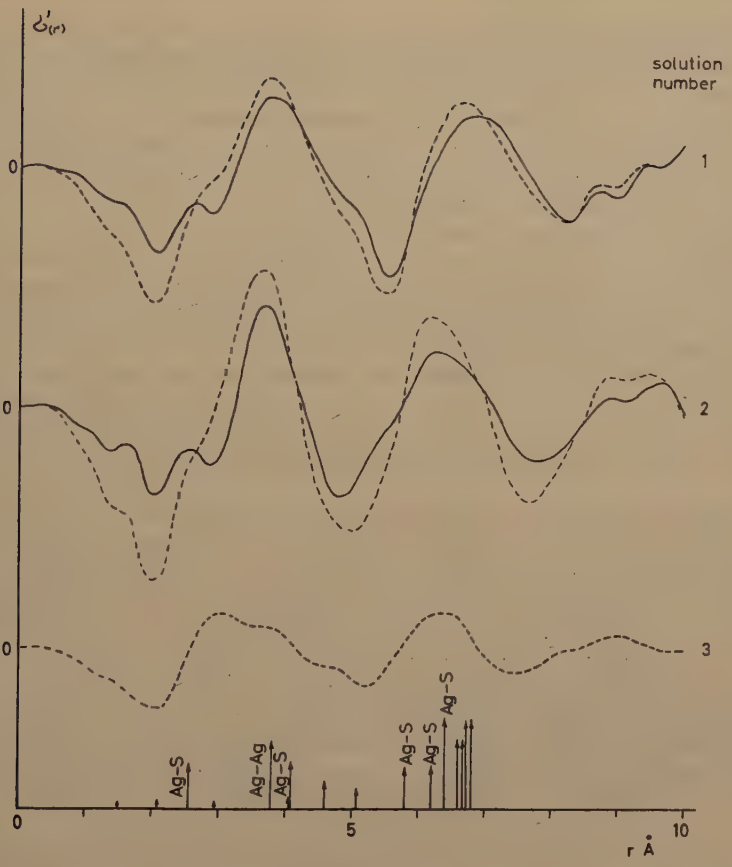

Fig. 12. Electron distribution functions in the thiosulphate system. The curves belonging to solutions 1 and 2 have been calculated for the same number of silver atoms. The dashed lines represent $\sigma'_{(r)}$ for the investigated solutions while the full-drawn ones give $\sigma'_{(r)\,\text{solution}} - \sigma'_{(r)\,\text{"solvent"}}$.

thiosulphate ions. The S–S distance 2.08 Å within the thiosulphate group cannot be found in the distribution functions which have a minimum here. The peaks at $\approx$2.5 Å correspond well to the sum of the covalent radii of silver and sulphur which shows that the silver atom is attached to the outer sulphur atom in thiosulphate and not to oxygen. The distance O–O within the thiosulphate group is also 2.5 Å but as the oxygen atoms are light compared with silver and sulphur the contribution from this distance is small. The distribution function of solution number 1 has maxima at 3.8 and 6.8 Å, a small superimposed peak at 5 Å and a minimum at 5.5 Å. It was tried to describe this function by different models of polynuclear ions but as the thiosulphate group contains five atoms it is difficult to get unambiguous results. If the outer sulphur in thiosulphate coordinates three silver atoms at normal tetrahedral angles the silver–silver distance should have been 4.3 Å. To explain the high peak at 3.8 Å it seemed necessary to assume that the silver–silver distance is 3.8 Å, which corresponds to a silver–outer sulphur–inner sulphur angle equal to 123°. Under this assumption different models were tried. The polynuclear

ions with the composition $Ag_m(S_2O_3)_{m+2}^{(m+4)-}$ may form layers consisting of condensed hexagons with three silver and three sulphur atoms in each ring (cf. Fig. 10). The silver–sulphur bonds may be directed towards the corners of a triangle with the silver atom in the center of the triangle (this idealized structure is represented by arrows in Fig. 12) or the silver atom may be in the center of a tetrahedron with a water molecule in the fourth corner. The polynuclear ions may also be built up from three-rings of silver placed over each other and joined by three thiosulphate groups between each pair of rings. The silver is also in this case placed in the center of a tetrahedron with a water molecule in the fourth corner. This model is in agreement with the result from the potentiometric measurements that three silver and three thiosulphate groups are added in each condensation step. The two last-mentioned models with a water molecule in the fourth corner of a tetrahedron also explain the water content in the crystals $NaAgS_2O_3 \cdot H_2O$ which precipitated from the supersaturated solutions in Ref. [25]. These models all seem to be able to explain the experimental distribution function of solution number 1 and it is consequently not possible to draw definite conclusions concerning the structure of the polynuclear ions from the experimental curve.

The maxima and minima of solution number 2 are shifted to the left compared with the ones belonging to solution number 1. Furthermore the peak at 3.7 Å is not as broad as the corresponding one in the upper curve. The structure of the ions thus seems to have been changed and this was also expected as solution number 2 has a higher ratio of thiosulphate to silver than solution number 1. This higher ratio will shift the equilibria towards mononuclear complexes.

It is interesting to note that the Ag–S distance in the thiocyanate solutions is longer than in the thiosulphate solutions. This effect may of course arise from the different character of the anions but it is also in agreement with the assumption that the silver atom in the thiocyanate solutions is coordinated to four sulphur atoms while it in the thiosulphate solutions is coordinated to less than four.

SUMMARY

The structure of silver complexes in water solutions of lithium iodide, ammonium thiocyanate, or sodium thiosulphate saturated with corresponding silver salts was studied by the aid of X-ray scattering.

In the iodide system the silver atoms are tetrahedrally surrounded by four iodine atoms at a distance of 2.9 Å. It has been proved that the tetrahedra are linked up to form polynuclear ions but it could not be established if the complex anions consist of chains or form discrete groups.

In the thiocyanate system the silver atoms are coordinated to the sulphur atom in the thiocyanate ion at the distance 2.6_5 Å. A peak at 4.3 Å shows that the silver atoms probably are tetrahedrally surrounded by thiocyanate ions.

In the thiosulphate system a peak at 1.5 Å corresponding to the sulphur–oxygen distance in the thiosulphate ion was observed. Further there was a peak at 2.5_5 Å corresponding to the silver–outer sulphur distance and one at 3.8 Å probably partly arising from the silver–silver distance.

The results obtained in this paper show that it is possible to evaluate the short distances between the heavy atoms in the ions in the solutions. It is not possible, however, to establish the complete structure of the polynuclear ions, especially not when the ligand ion contains more than one atom. An advanced experimental technique, giving refined intensity data, could perhaps give further information about the structure of the polynuclear ions.

The author wishes to thank Professor C. Brosset for the great interest he has shown in this work

and for valuable advice. He is also indebted to Sivilingeniör J. Krogh-Moe and Tekn. lic. U. Magnusson for the discussions held with them on various aspects on this problem and to Ing. L.-E. Marthén for making the exposures. A grant from *Statens Tekniska Forskningsråd* made this work possible.

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Tryckt den 29 april 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 45

Communicated 8 January 1958 by Arne Fredga and Karl Myrbäck

3-Substituted thiophenes

3-Thienylsubstituted ketones

By Salo Gronowitz

With 10 figures in the text

In connection with other work (1) it was important to have convenient methods for preparing 3-thienylsubstituted ketones. Unlike the 2-isomers, which are easily available through Friedel-Crafts' synthesis (2) or the H_3PO_4 (3) catalyzed acylation of thiophene, only a few 3-thienylsubstituted ketones were known until a few years ago. Steinkopf and Schmidt (4) prepared 3-(3′-thenoyl)-thiophene in low yield through the dry distillation of the calcium salt of 3-thiophenecarboxylic acid and Hoch (5) obtained ethyl 3-thienylketone in low yield through the reaction of ethylcyanide with 3-thiophenemagnesium bromide, obtained together with a large amount of ethyl magnesiumbromide in the entrainment grignardation of 3-bromothiophene.

Recently, however, Campaigne and Thomas (6) prepared 3-thienylsubstituted ketones through the reaction of 3-thenoyl chloride with organocadmium compounds. They (7) prepared 3-thiophenecarboxylic acid through side-chain bromination of 3-methylthiophene, conversion of the bromomethyl compound to the aldehyde according to Sommelet, and oxidation of the aldehyde to the acid with silver oxide. The present author (8) has found, however, that 3-thiophenecarboxylic acid is most conveniently prepared from 3-thienyllithium, so investigations were started to utilize the metalorganic reagent directly for ketone synthesis. The most obvious method was the reaction between the organolithium compound and nitriles. Especially as Swain (9) found that phenyllithium reacted with benzonitrile over a hundred times faster than phenyl magnesiumbromide it was hoped that the reaction could be carried out at low temperature where 3-thienyllithium is stable. Gilman *et al.* (10) have also shown that the reaction between phenyllithium and benzonitrile is completed in less than 5 minutes at $-20°$. This method is, however, not very suitable for the reaction with nitriles having α-hydrogens, especially not acetonitrile. In order to obtain good yields with Grignard reagents and acetonitrile a large excess of the metalorganic reagent has been used. Sumrell (11) recently investigated the reaction of organolithium reagents with nitriles having α-hydrogens. The yields when 10 % excess of organolithium reagent was used, however, were not better than with the Grignard reagents: about 30 %. It was hoped that at $-70°$, addition across the $-C\equiv N$ bond would be favoured over the metalation of the α-hydrogen in the nitrile.

This was however not the case. When 3-thienyllithium was reacted with acetonitrile at $-70°$, only very small amounts of 3-acetothienone could be obtained. Under the same conditions a 75 % yield of 3-benzothienone was obtained with benzonitrile. Campaigne and Thomas (6) have obtained a 56 % yield through reacting 3-thenonitrile with a five-fold excess of phenyl magnesiumbromide.

As a convenient route to aliphatic 3-thienylketones, especially 3-acetothienone, a reversal of the reagents used by Campaigne and Thomas (6), that is to use 3-thienylcadmium and aliphatic acid chlorides, seemed promising, as it has been found (12) in the synthesis of propiophenone that the same yields are obtained from diethylcadmium and benzoyl chloride as from diphenylcadmium and propionyl chloride. Gilman and Nelson (13) in their paper introducing the use of organocadmium compounds mention briefly that organolithium compounds can be used instead of Grignard reagents for the preparation of cadmium compounds and state that they obtained acetophenone from phenyllithium, cadmium chloride and acetyl chloride without giving any details. In reviews by Cason (14) and by Shirley (15) on the use of organocadmium compounds the same statement appears. However, they give no additional references to cases where lithium compounds have been used for the preparation of cadmium compounds. The present author found, however, that when 3-thienyllithium was reacted with $CdCl_2$ a negative Michler's ketone test (16) was obtained only after about 12 hours' reflux. Similar results were obtained with 2-thienyllithium and phenyllithium.

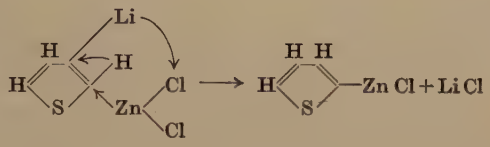
When phenyllithium or 2-thienyllithium were refluxed with $CdCl_2$ until the Michler's ketone test was negative about 65 % of acetophenone and 57 % 2-acetothienone could be obtained upon reaction with acetyl chloride. With 3-thienyllithium, however, extensive decomposition had occurred and only 15 % of an impure acetothienone fraction was obtained. If acetyl chloride was added before Michler's ketone test was negative only traces of ketones were obtained as could be expected. From phenyllithium, 1.1-diphenylethylene could be isolated. In the case of the thiophene analogues the residue polymerized when distillation in vacuo was attempted. In order to show that the slow reaction between the organolithium compounds and $CdCl_2$ did not depend upon the quality of the $CdCl_2$ and ether used, parallel experiments were carried out with phenyl magnesiumbromide and 2-thienyl magnesiumbromide obtained from bromobenzene and 2-bromothiophene respectively. In these cases the Michler's ketone test was negative after 20 minutes of reflux and with acetyl chloride 70 % and 55 % respectively of the ketones were obtained. (These yields should not be considered as the optimal as no efforts were made to enhance the yield by changing the solvent to benzene before adding the acid chloride.)

It is thus apparent that organolithium compounds react slower than the corresponding Grignard reagents with cadmium chloride. The reason for this is not quite clear. It might depend upon trivial things like the difference in solubility of cadmium chloride in the different reaction media. A closer study of the literature also revealed that Cason and Wordie (17) have had some difficulties in obtaining the cadmium reagent from 1-naphthyllithium, and 8-methyl-1-naphthyllithium. This appears to be the only investigation besides that of Gilman and Nelson (13) in which lithium compounds have been used for the preparation of the corresponding cadmium compounds. However, when phenyllithium was reacted with a solution of zinc chloride in ether, Michler's ketone test became negative at once and after addition of acetyl chloride 80 % of acetophenone was ob-

tained. It is thus much more convenient to transform an organolithium compound into the corresponding zinc compound when an organometal compound of lower reactivity is needed.

When 3-thienyllithium was reacted with $ZnCl_2$ and acetyl chloride an interesting isomerization occurred and 55 % of a mixture of 2- and 3-acetothienone was obtained. IR-analysis of this mixture showed it to consist of 65 % 2-acetothienone and 31 % of 3-acetothienone.

As 3-thienyllithium is stable under the conditions used the rearrangement may have occurred during the reaction of zinc chloride with 3-thienyllithium or during the following reaction with acetyl chloride. The following concerted mechanism may be feasible:



$ZnCl_2$ attacks at the 2-carbon and in a concerted reaction the proton shifts to the 3-position. It may also be possible that the rearrangement occurs during the last step of the reaction and that besides the normal reaction in the 3-position $ZnCl_2$-catalyzed acylation occurs in the 2- or 5-position.

There are, however, some results (*vide infra*) which indicate that the rearrangement occurs in the last step. This question thus becomes intimately connected with the nature of the $ZnCl_2$-catalyzed acylation of thiophene discovered by Hartough and Kosak (18) the mechanism of which is not known in detail.

Newman (19) has worked out a convenient ketone synthesis by reacting Grignard reagents with acid anhydrides at low temperature. When this reaction was applied to 3-thienyllithium the reaction did not stop completely at the ketone stage, and only a small yield of 3-acetothienone was obtained. The success of this synthesis according to Newman depends upon the formation of an insoluble complex, which arrests the reaction at the ketone stage. Such an insoluble complex did not appear in the case of the lithium compound and it is possible that this, besides the greater reactivity of the lithium compounds, is the reason for the inapplicability of the lithium reagent.

It was, however, found that when 3-thienyllithium first was converted to the Grignard reagent with $MgBr_2$-etherate and then reacted with acetic anhydride at $-70°$ 3-acetothienone free from the 2-isomer was obtained in good yield. It is thus interesting to note that no rearrangement occurs in this reaction. If one makes the plausible assumption that the reaction mechanism for the reaction of organic lithium compounds with $ZnCl_2$ and $MgBr_2$ is similar this would indicate that the isomerization in the earlier discussed reaction occurs in the last step and that $ZnCl_2$ transfers 3-thienyllithium into 3-thienyl zinc chloride.

It is thus possible in this way to obtain a Grignard reagent from 3-bromothiophene free from entrainment Grignard reagent. Recent publications by Normant (20) and Ramsden (21) describe the preparation of Grignard reagents from unreactive halides such as chlorobenzene, in tetrahydrofuran. It was, however, found that 3-bromothiophene which in ether gives the Grignard reagent only through the entrainment reaction (22), also reacted very sluggishly in tetrahydro-

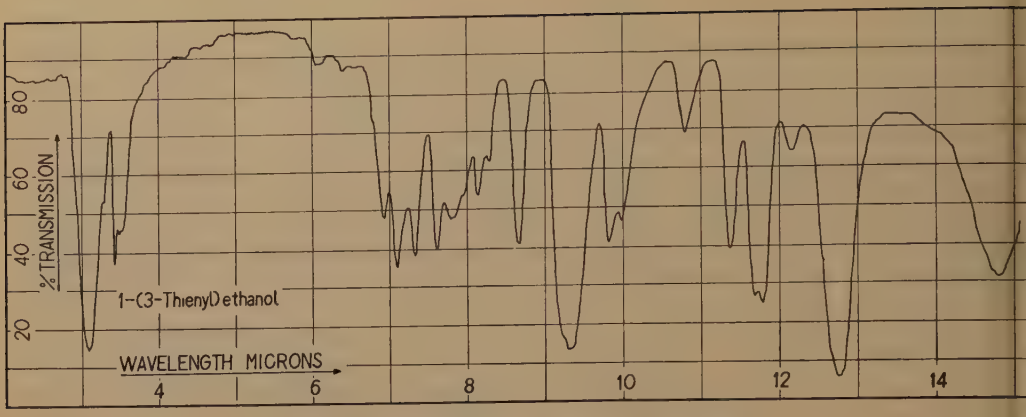


Fig. 1. IR-spectrum of 1-(3-thienyl)-ethanol.

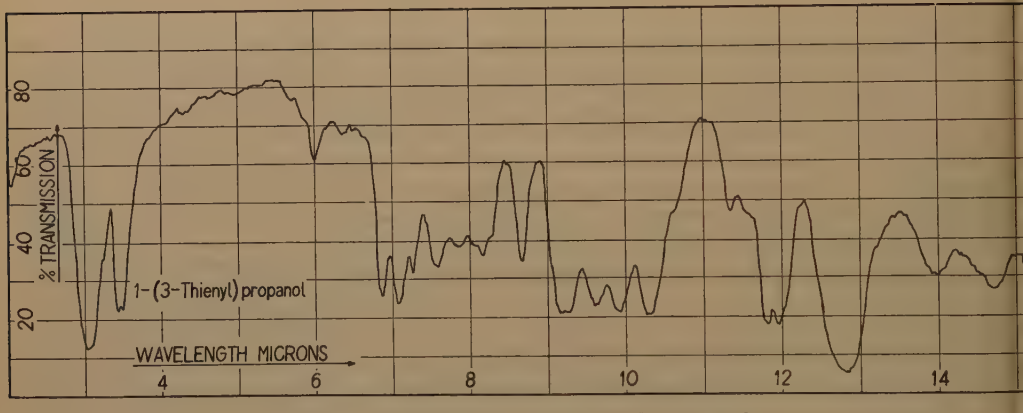


Fig. 2. IR-spectrum of 1-(3-thienyl)-propanol.

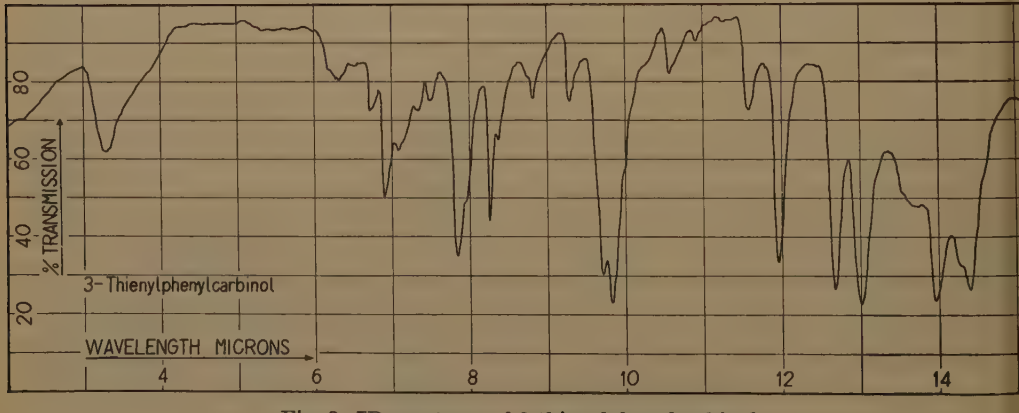


Fig. 3. IR-spectrum of 3-thienylphenylcarbinol.

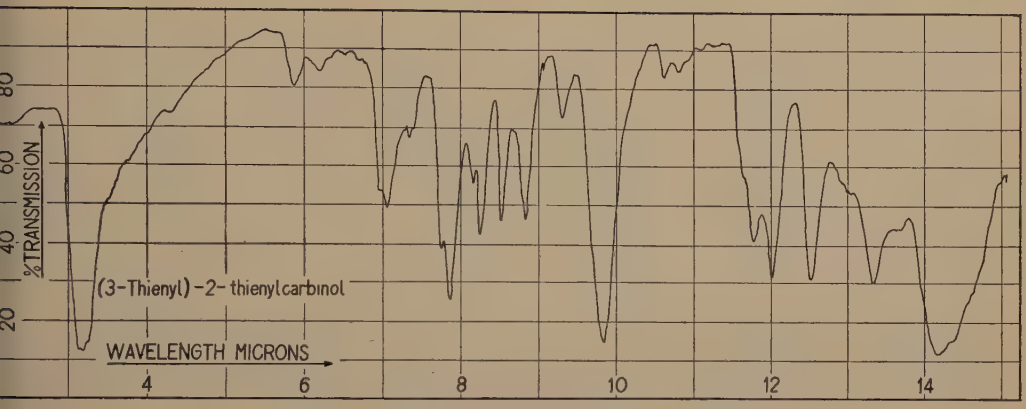


Fig. 4. IR-spectrum of (3-thienyl)-2-thienylcarbinol.

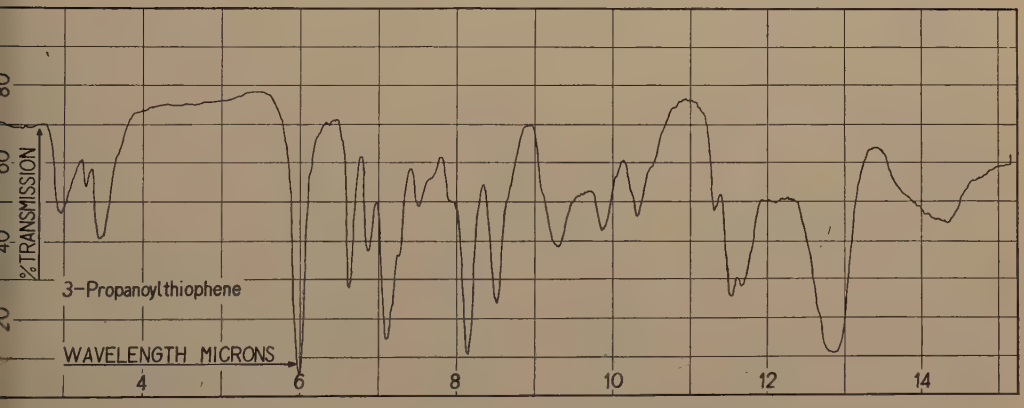


Fig. 5. IR-spectrum of 3-propanoylthiophene.

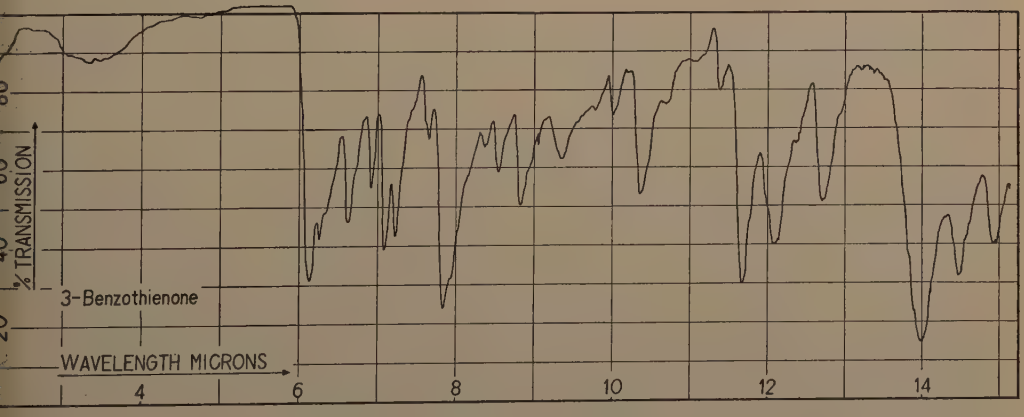


Fig. 6. IR-spectrum of 3-benzothienone.

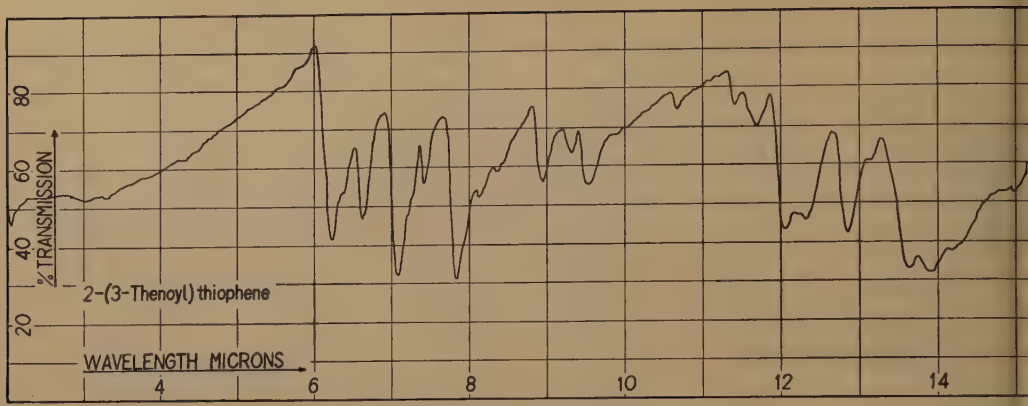


Fig. 7. IR-spectrum of 2-(3-thenoyl)-thiophene.

Table 1. 3-Thienylsubstituted carbinols and ketones from 3-thienyllithium.

Aldehyde used	Alcohol formed	Yield %	Ketone formed	Yield %
Acetaldehyde	1-(3-thienyl)-ethanol	74	3-acetothienone	75
Propionic aldehyde	1-(3-thienyl)-propanol	75	3-propanoylthiophene	67
Benzaldehyde	(3-thienyl)-phenylcarbinol	79	3-benzoylthiophene	77
2-Thiophenecarboxaldehyde	(3-thienyl)-2-thienylcarbinol	77	2-(3-thenoyl)-thiophene	72

furan. After 10 hours most of the magnesium had not reacted. Upon carbonation only 10 % of impure 3-thiophenecarboxylic acid was obtained and most of the bromothiophene could be recovered. The slow reaction could not depend upon the quality of reagents used, as in parallel experiments with chlorobenzene Ramsden's (21) results could be confirmed.

3-Thienylsubstituted ketones were also obtained through CrO_3-oxidation of 3-thienylsubstituted carbinols in high yields. These practically unknown compounds are easily available through the reaction of 3-thienyllithium with aldehydes at $-70°$. The results obtained are shown in Table 1. The IR-spectra of some 3-thienylsubstituted alcohols and ketones are given in Figures 1–7.

Experimental

All experiments in which metalorganic compounds were used were carried out in a nitrogen atmosphere.

3-Benzothienone.—18.1 g (0.176 moles) of benzonitrile in 30 ml of ether was added dropwise at $-70°$ to a solution of 3-thienyllithium (8), prepared at the same temperature from 25.3 g (0.155 moles) of 3-bromothiophene in 50 ml of ether and 135 ml 1.25-*N* *n*-butyllithium (23). The cooling was continued for 45 minutes. The cooling-bath was then removed and when the temperature had risen to $-10°$ (about 2 hours), the mixture was cautiously decomposed with ice-water and 2-*N* HCl. The ether phase was separated and the acid phase refluxed for 20 minutes

in order to hydrolyze the ketimine hydrochloride. After cooling, the organic layer which separated was taken up in ether and the acid phase refluxed for an additional hour, cooled and extracted with ether. The combined ether phase was washed with dilute sodium carbonate solution and water, dried and fractionated, yielding 22.6 g (75 %) of 3-benzothienone, b.p. 125–127°/1 mm Hg. M.p. 63–64° after one recrystallization from methanol. 2.4-Dinitrophenylhydrazone, m.p. 212–213°; Campaigne and Thomas (6) give 63–64° and 211–212° respectively.

Anal. $C_{11}H_8OS$ (188.2):
calc.: C 70.18 H 4.28 S 17.03,
found: C 69.85 H 4.27 S 17.10.

When in the same manner 25.0 g (0.153 moles) 3-bromothiophene 130 ml 1.25-*N* *n*-butyllithium and 6.15 g (0.150 moles) of acetonitrile were reacted only 2.9 g of a fraction, b.p. 90–110°/10 mm Hg, $n_D^{25} = 1.4822$, was obtained. This fraction partly crystallized when inoculated with a crystal of 3-acetothienone and after one recrystallization from petroleum ether 0.85 g of impure 3-acetothienone m.p. 48–53° was obtained.

Cadmium compounds from lithium compounds.—To a solution of 0.18 moles of phenyllithium in 150 ml of absolute ether was added 25 g (0.14 moles) $CdCl_2$. After 12 hours' reflux Michler's ketone test was negative. After one hours' reflux additional small amounts of $CdCl_2$ were added according to the recommendations of Gilman and Nelson, but without effect. The mixture was cooled in ice-water and 12.0 g (0.15 moles) of freshly distilled acetyl chloride in 20 ml of dry ether was added. The mixture was then refluxed for one and a half hour. After cooling the reaction mixture was decomposed with ice and dilute sulphuric acid and worked up in the usual manner. Upon fractionation 11.7 g (64 %) of acetophenone, b.p. 85–88°/14 mm Hg, $n_D^{25} = 1.5312$, was obtained.

In a similar experiment with 2-thienyllithium prepared through metalation of 21.1 g (0.25 moles) of thiophene in 50 ml of ether with 200 ml of a 1.25-*N* solution of *n*-butyllithium, a negative Michler's ketone test was obtained after 12 hours' reflux with 35.5 g of $CdCl_2$, 10 g of which was added in 5 g portions after two and four hours' reflux. When the colour test was negative 15.2 g (0.194 moles) of acetyl chloride in 30 ml of ether was added dropwise and worked up as above 13.8 g (51 %) of 2-acetothienone, b.p. 93–95°/13 mm Hg, $n_D^{25} = 1.5588$, was obtained.

In the experiment with 3-thienyllithium the following modification was used: From 180 ml 1.35-*N* *n*-butyllithium and 32.0 g (0.20 moles) of 3-bromothiophene in 50 ml of dry ether a solution of 3-thienyllithium was prepared in the usual manner at −70°. This solution was under nitrogen transferred to another four-necked flask, equipped with stirrer, reflux condenser with calcium chloride tube, dropping funnel and nitrogen inlet, in which 30 g (0.16 moles) of $CdCl_2$ and 100 ml of dry ether were placed. After 12 hours' reflux, Michler's ketone test was negative, and 17.0 g (0.217 moles) of freshly distilled acetyl chloride was added under cooling, the procedure was then continued as above. On distillation 4.5 g (18 %) of a fraction, b.p. 86–89°/10 mm Hg, $n_D^{25} = 1.5615$, was obtained. Its IR-spectrum contained the peaks of both 2- and 3-acetothienone. The residue polymerized and decomposed upon attempted distillation.

Cadmium compounds from magnesium compounds.—These reactions were performed according to Gilman and Nelson (13). When to the Grignard reagent from 30 g (0.18 moles) 2-bromothiophene and 4.6 g (0.19 moles) of magnesium in 200

ml of ether 20.0 g (0.11 moles) of $CdCl_2$ was added, Michler's ketone test was negative after 20 minutes. Upon addition of 12.0 g (0.15 moles) acetyl chloride, 10.2 g (53 %) of 2-acetothienone, b.p. 96–99°/13 mm Hg, $n_D^{25} = 1.5635$, was obtained.

Zinc compounds from lithium compounds.—To a solution of 0.19 moles of phenyllithium in 175 ml of ether 20 g (0.145 moles) of $ZnCl_2$ in absolute ether, prepared with the precautions given by Shirley (15) was added as rapidly as the refluxing mixture allowed. Immediately after the addition of the $ZnCl_2$-solution Michler's ketone test was negative. Ether was then distilled from the reaction mixture until its volume was about 150 ml. 12.5 g (0.16 moles) of acetyl chloride in 100 ml of dry benzene was added under cooling. After one hour's reflux the mixture was cooled, hydrolyzed with ice and dilute hydrochloric acid. The organic phase was washed with sodium carbonate solution and water and dried. Upon fractionation 15.6 g (81 %) of acetophenone, b.p. 83–87°/12 mm Hg, $n_D^{25} = 1.5320$, was obtained.

A solution of 3-thienyllithium prepared from 300 ml 0.95-*N* *n*-butyllithium in ether and 40.0 g (0.25 moles) of 3-bromothiophene at −70° under nitrogen was rapidly added to a solution of 28 g (0.205 moles) of $ZnCl_2$ in ether. Ether was then distilled from the reaction mixture until the volume was about 200 ml. Then under cooling 19.0 g (0.24 moles) of acetyl chloride in 100 ml benzene was added and the work continued as above. 16.3 g (55 %) of a fraction, b.p. 96–100°/15 mm Hg, $n_D^{25} = 1.5608$, was obtained. The IR-spectrum of this fraction contained the peaks of both 2- and 3-acetothienone (Fig. 9). Fig. 8 and 10 show the spectra of 2- and 3-acetothienone.

For the analysis of this mixture the peak at 10.73 μ was used for the 2-isomer and the peak at 11.12 μ for the 3-isomer. The analysis was performed according to the base-line method (24) and a calibration curve was constructed from known mixtures in carbon disulphide. It was found that the crude mixture obtained contained 65 % of 2-acetothienone and 31 % 3-acetothienone.

After several crystallizations from petroleum ether 1.5 g of almost pure 3-acetothienone, m.p. 55–57°, could be isolated.

3-Thienyllithium and acetic anhydride.—3-Thienyllithium was prepared at −70° from 170 ml 1.35-*N* *n*-butyllithium acid and 33.5 g (0.205 moles) of 3-bromothiophene in 50 ml ether. This solution, kept at this temperature, was forced by nitrogen pressure in small portions into a dropping funnel through a glasstube extending to the bottom of the reaction flask. The dropping funnel (which preferably should be double-walled and cooled with Dry Ice-acetone) was connected to the usual four-necked flask in which 70.0 g of acetic anhydride in 200 ml of absolute ether was cooled to −70°. The solution of 3-thienyllithium was in this manner added dropwise to the cooled acetic anhydride. When all the thienyllithium had been added, the mixture was stirred at −70° for 2 hours. The cooling bath was then removed, and after 2 hours, the mixture was hydrolyzed with water and the ether phase extracted with sodium carbonate solution until all the acetic acid was removed (6–8 times). The combined alkaline phases were then extracted with ether. The dried ether phase gave upon distillation 5.5 g (21 %) of 3-acetothienone, b.p. 88–90°/11 mm Hg, which crystallized in the condensor (m.p. 58–59° after recrystallization from petroleum ether). Its IR-spectrum showed it to be free from the 2-isomer. 4.7 g of a fraction boiling 146–150°/11 mm Hg, which could be 1.1-di-(3-thienyl)-ethylene, and a residue of 6.5 g which started to decompose on attempted distillation were not further investigated.

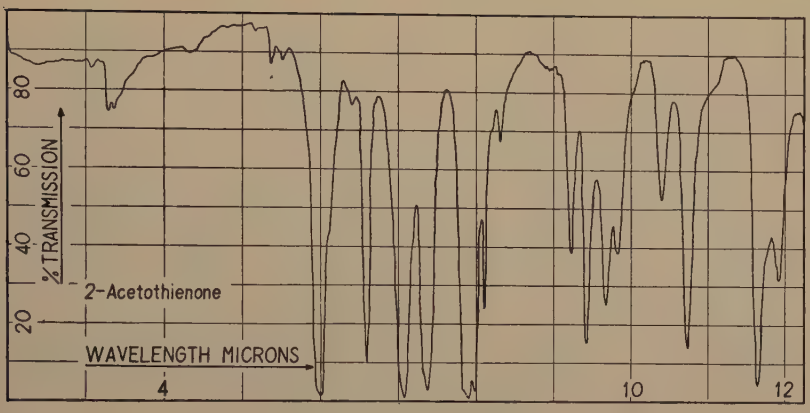


Fig. 8. IR-spectrum of 2-acetothienone in CCl_4.

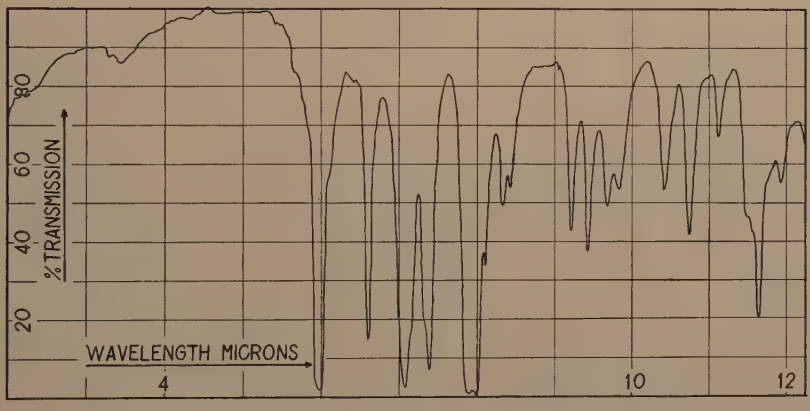


Fig. 9. IR-spectrum of product from the reaction of 3-thienyllithium, $ZnCl_2$ and acetyl chloride in CCl_4.

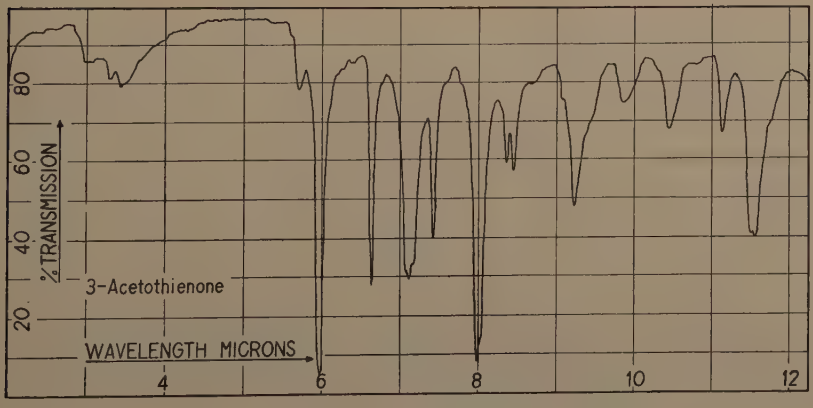


Fig. 10. IR-spectrum of 3-acetothienone in CCl_4.

In spite of several other experiments the yield could not be increased, but stayed between 15–20 %.

3-Thienyllithium, magnesiumbromide and acetic anhydride.—A solution of 3-thienyllithium prepared as usual at −70° from 200 ml 1.35-*N* *n*-butyllithium and 37.5 g (0.23 moles) of 3-bromothiophene was as above forced over into a dropping-funnel with nitrogen and added in a slow stream to $MgBr_2$ in ether. The magnesium bromide was prepared by slowly dropping 45 g (0.28 moles) of bromine into an ice-water-cooled stirred mixture of 10 g magnesium in 150 ml of dry ether and 5 ml dry benzene under nitrogen. (The benzene is added to prevent the magnesiumbromide etherate from crystallizing out.) The two layer system obtained was decanted from excess magnesium. After the addition of the 3-thienyllithium a single clear solution was obtained. This solution of 3-thiophene magnesiumbromide (or better 3-thienylmagnesium and $MgBr_2$) (25) was cooled to −70° after half an hour and in the same manner as described above added dropwise during 90 minutes to a solution of 55 g freshly distilled acetic anhydride in 200 ml of ether which also was cooled to −70°. A heavy white precipitate was formed and after 2 hours' stirring at −70°, the cooling bath was removed. When the temperature had risen to −10°, the mixture was hydrolyzed with ammonium chloride solution and worked up as in the preceding experiment. 19.7 g (68 %) of 3-acetothienone, b.p. 88–89°/11 mm Hg, m.p. 57.5–58.5°, free from the 2-isomer was obtained.

Grignard reaction of 3-bromothiophene in tetrahydrofuran.—To 6.0 g (0.25 moles) of magnesium were added 3 g of ethyl bromide and 20 ml of a solution of 40 g (0.25 moles) of 3-bromothiophene in 50 ml of tetrahydrofuran (dried over sodium hydroxide, sodium, and distilled from $LiAlH_4$ before use). A spontaneous reaction started and the remaining 3-bromothiophene was added during 30 minutes. Heat was applied in order to keep the reaction mixture at reflux temperature. It was then refluxed for ten hours and then carbonated with Dry Ice. 4.9 g of unused Mg was left and from the neutral phase 27.2 g of 3-bromothiophene was recovered. From the acid fraction, 1.9 g of impure 3-thiophenecarboxylic acid (85–135°), which was not further purified, was obtained.

In a parallel experiment with chlorobenzene all the magnesium was consumed after two hours' reflux.

1-(3-Thienyl)-ethanol.—To a solution of 3-thienyllithium prepared at −70° from 160 ml 1.4-*N* *n*-butyllithium and 31.0 g (0.19 moles) of 3-bromothiophene was added dropwise 10.6 g (0.24 moles) of acetaldehyde in 50 ml of ether. 30 minutes after the addition the cooling bath was removed and after an hour the solution was hydrolyzed. The ether phase was washed with water, dried and fractionated. 17.9 g (74 %), b.p. 70–72°/1.5 mm Hg, $n_D^{25} = 1.5418$, of 1-(3-thienyl)-ethanol was obtained. Troyanowsky (26) gives b.p. 98°/12 mm Hg, $n_D^{14} = 1.549$.

Phenylurethane.—M.p. 82–83°.

Anal. $C_{13}H_{13}NO_2S$ (247.3):

calc.:	C 63.13	H 5.30	N 5.66	S 12.96,
found:	C 63.25	H 5.41	N 5.68	S 12.88.

1-(3-Thienyl)-propanol.—From 160 ml 1.5-*N* *n*-butyllithium 36 g (0.221 moles) 3-bromothiophene and 14.2 g (0.245 moles) of propionic aldehyde 23.5 g (75 %) 1-(3-thienyl)-propanol, b.p. 78–80°/1.5 mm Hg, $n_D^{25} = 1.5250$, was obtained.

Anal. $C_7H_{10}OS$ (142.2):
calc.: C 59.12 H 7.09 S 22.55,
found: C 59.18 H 7.15 S 21.99.

The somewhat low sulphur value may indicate that the substance is only 98–99 % pure.

Phenylurethane.—M.p. 89–90°.

Anal. $C_{14}H_{15}NO_2S$ (261.3):
calc.: C 64.34 H 5.79 N 5.36 S 12.27,
found: C 64.45 H 5.86 N 5.37 S 12.11.

3-Thienylphenylcarbinol.—135 ml 1.5-*N* *n*-butyllithium 30.5 g (0.187 moles) 3-bromothiophene and 22.0 g (0.207 moles) of benzaldehyde were reacted and worked up as above. Ether and butylbromide were distilled off, the latter in vacuo, and the residue recrystallized from petrol (b.p. 60–85°). 28.2 g (79 %) of 3-thienylphenylcarbinol, m.p. 90–90.5°, was obtained in white fine needles. The carbinol can also be recrystallized from aqueous ethanol.

Anal. $C_{11}H_{10}OS$ (190.3):
calc.: C 69.44 H 5.30 S 16.85,
found: C 69.53 H 5.30 S 16.86.

3-Thienyl-2-thienylcarbinol.—From 150 ml 1.4-*N* *n*-butyllithium 32.0 g (0.196 moles) 3-bromothiophene and 24.6 g (0.220 moles) 2-thiophenecarboxaldehyde 29.5 g (77 %) of the carbinol, m.p. 67.5–68.5°, was obtained after recrystallization from petrol (60–85°).

Anal. $C_9H_8OS_2$ (196.3):
calc.: C 55.06 H 4.11 S 32.67,
found: C 55.21 H 4.11 S 32.56.

3-Acetothienone.—To 10.0 g (0.078 moles) of 1-(3-thienyl)-ethanol in 75 ml of acetic acid was dropped during one hour 5.3 g (0.053 moles) of CrO_3 dissolved in 30 ml water and 70 ml acetic acid. The temperature was held between 25–30°. It was stirred for an additional hour at room temperature, after which the dark violet solution was poured in 1 l water. The aqueous phase was extracted three times with ether, the combined ether phases were extracted with sodium carbonate solution until the extract was alkaline, and finally the alkaline phase was extracted once with ether. The ether phase was dried and upon distillation 7.1 g (71 %) of 3-acetothienone, b.p. 106–107°/25 mm Hg, was obtained. M.p. 58–59° after recrystallization from petroleum ether.

3-Propiothienone.—In the same manner 6.7 g (67 %) of 3-thienyl ethyl ketone was obtained from 10.0 g (0.0703 moles) of carbinol in 75 ml acetic acid and 4.8 g (0.048 moles) of CrO_3 in 30 ml H_2O and 70 ml acetic acid. B.p. 95–97°/81 mm Hg, $n_D^{25} = 1.5412$. Semicarbazone m.p. 186–188°. Campaigne and Thomas (6) give $n_D^{18} = 1.5471$. Semicarbazone m.p. 186–187°.

3-Benzothienone.—10.0 g (0.0525 moles) 3-thienylphenylcarbinol in 75 ml acetic acid and 3.6 g (0.036 moles) CrO_3 in 30 ml water and 70 ml acetic acid gave 75 g (75 %) 3-thienylphenylketone, b.p. 127–130°/1 mm Hg. After one recrystallization from methanol white plates, m.p. 63–64°, were obtained which gave no melting-point depression with the ketone obtained in the reaction with benzonitrile.

2-(3-Thenoyl)-thiophene.—From 10.0 g (0.0509 moles) 3-thienyl-2-thienylcarbinol in 75 ml acetic acid and 3.5 g (0.035 moles) CrO_3 in 30 ml H_2O and 70 ml acetic acid were obtained, after removal of ether, 7.2 g (72 %) of 2-(3-thenoyl)-thiophene. After recrystallization from methanol white needles, m.p. 62–63°, were obtained. Campaigne and Thomas (6) give m.p. 64°.

Anal. $C_9H_6OS_2$ (194.3):

calc.:	C 55.64	H 3.11	S 33.01,
found:	C 55.97	H 3.07	S 29.91.

All melting-points were determined in a Kofler hot-stage microscope. A Perkin Elmer model 21 spectrophotometer equipped with sodium chloride prism was used for the IR absorption spectra. The solid substances were examined as solid pressed potassium bromide plates.

SUMMARY

It has been found that, contrary to earlier statements, organolithium compounds react only sluggishly with $CdCl_2$. They can, however, smoothly be converted to zinc compounds which can be utilized for ketone synthesis with acid chlorides. When this reaction was applied to 3-thienyllithium, rearrangement to the 2-isomer occurred to a large extent and an isomer mixture was obtained. It was, however, found that 3-acetothienone could be synthesized from 3-thienyllithium in good yields by converting 3-thienyllithium into the Grignard compound with $MgBr_2$ and then reacting with acetic anhydride at low temperature. The reaction between 3-thienyllithium and $MgBr_2$ constitutes a useful route to obtain 3-thiophene magnesiumbromide free from entrainment Grignard compound. 3-Thienylsubstituted ketones were also obtained through the reaction of 3-thienyllithium with nitriles and through chromic acid oxidation of the corresponding alcohols which are conveniently obtained through the reaction of 3-thienyllithium with aldehydes.

ACKNOWLEDGEMENTS

The author is greatly indebted to Professor Arne Fredga for valuable discussions. Thanks are also due to the head of this institute, Professor Nils Hellström, for his kind interest and for all facilities put at the author's disposal. The IR-spectra were recorded by Mr. Stig Bergvall and the microanalysis by Dr. A. Bengtsson of the Analytical Department of the Chemical Institute, University of Uppsala.

Institute of Organic Chemistry, Royal Agricultural College, Uppsala, December 1957.

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Tryckt den 3 juni 1958.

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 46

Read 12 February 1958

Studies on synthetic growth substances

X. The enantiomorphs of benznorcaradiene-carboxylic acid

By Arne Fredga

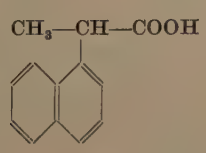
With 1 figure in the text

It is well known that a substance showing auxin activity must fulfil special structural conditions. A very important class of such substances possesses a basal ring system showing surface activity and a group of acid character in a certain spatial position to the ring system (1). According to Veldstra, the acid group must not lie in the plane of the ring (2). A typical example is the 1-naphthyl-acetic acid, which has been investigated by numerous authors.

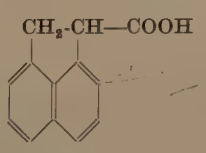
During recent years, stereochemical aspects have attracted considerable interest. The optically active forms of the α-(1-naphthyl)-propionic acid (I), the next higher homologue of the 1-naphthyl-acetic acid, were prepared and examined some years ago (3, 4). In several tests, the two forms have the same biological activity, but in others there was a marked difference.

The racemic acenaphthene-7-carboxylic acid (II) has been investigated by Julia, who found a considerable auxin activity (5). As this acid has a certain structural likeness to I, it would be of great interest to test the optical antipodes. Preliminary experiments by the present author indicate that the acid can be resolved rather easily using brucine and cinchonine, but the resolution has not yet been carried out on a preparative scale.

A somewhat different stereochemical aspect is presented by cinnamic acid (III). It has long been known that the *cis*-form has a positive auxin effect (6),



I



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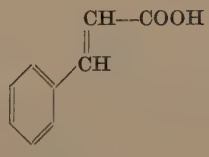

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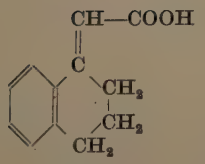

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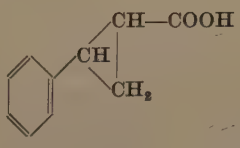
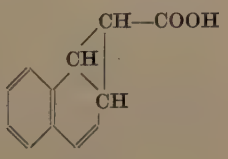


V



VI

while the *trans*-form is inactive or even an anti-auxin (7). Havinga and Nivard found the same effects for *cis*- and *trans*-forms of other unsaturated acids (8), e.g. the 1:2:3:4-tetrahydronaphthylidene-1-acetic acid (IV). In the literature it is sometimes suggested that the carboxyl group of this acid must lie in the ring plane. The aforenamed authors and later Thimann (9) have pointed out, however, that the right-hand ring in the formula must be buckled. In such a case, the carboxyl group cannot be located in the plane of the aromatic ring, but of course it may be difficult to estimate the deviation.

In 1951, Veldstra and van de Westeringh studied the *cis*- and *trans*-phenylcyclopropane-carboxylic acids (V) and found similar differences (10). In this case, both forms may be resolved into optical antipodes, but only the racemic acids were investigated.

In the course of current investigations on plant growth regulators, the present author has studied the benznorcaradiene-carboxylic acid (VI) obtained from naphthalene and ethyl diazoacetate (11). It contains three asymmetric carbon atoms, but as the cyclopropane ring is most probably fused to the six-membered ring in *cis*-position, the number of possible stereoisomers is reduced. Two geometrical isomers should exist, which may be called the *cis*- and *trans*-form (Fig. 1), and both are racemic. The number of optically active forms must thus be four.

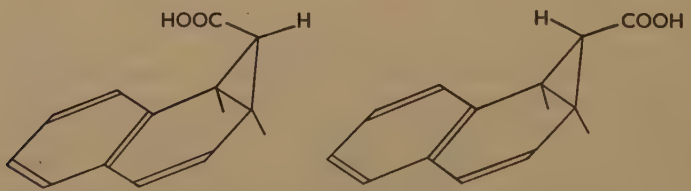


Fig. 1.

Only one racemic form is known as yet, and it has now been resolved into the optical antipodes using α-phenyl-ethylamine and β-phenyl-*iso*propylamine. The growth-regulating properties have been studied by Professor Börje Åberg, Royal Agricultural College, Uppsala, who has kindly reported some preliminary results. In the tests used (wheat root, flax root and Avena coleoptile test) both forms were found to be anti-auxins. There is, however, an obvious difference between the enantiomorphs, the (−)-form having a stronger and more typical anti-auxin effect.

As stated above, the *trans*-forms of cinnamic acid (III) and phenylcyclopropane-carboxylic acid (V) are both anti-auxins, whereas the corresponding *cis*-forms have positive auxin effect. In the location of the carboxyl group relative to the aromatic ring these *trans*-acids have a certain likeness to the *trans*-form of the benznorcaradiene-carboxylic acid. This may be an indication that the acid investigated is really the *trans*-form.

This attribution seems likely also for other reasons. The *trans*-form can be expected to be more stable than the *cis*-form. As the synthesis of the acid from naphthalene and ethyl diazoacetate is performed at a rather high temperature (140–150°), it is most probable that the product obtained represents the more

stable isomeride. In such case it may be very difficult, perhaps impossible, to obtain the *cis*-form.

It should be mentioned that a number of irradiation experiments have been carried out using UV-light of wavelengths absorbed by the acid. No indication for a simple steric rearrangement could be observed, but only far-reaching destruction of the molecule.

Experimental

The *racemic acid* was obtained by the courtesy of Dr. Gottfried Juppe, Munich, Germany.

Resolution of the racemic acid. Preliminary experiments with quinine and cinchonidine gave readily crystallising salts but very poor resolution. The salts of strychnine, brucine and cinchonine could not be obtained as crystals. Good resolution was obtained with α-phenyl-ethylamine and β-phenyl-*iso*propylamine, both yielding an acid with a direction of rotation opposite to that of the base used. Both bases were used in the final resolution and consistent values of the maximum rotation were obtained.

16.7 g acid (0.09 mole) and 12.1 g (0.09 mole) of (−)-β-phenyl-*iso*propylamine were dissolved in 70 ml methanol. The salt was filtered off after standing for 24 hours and recrystallised from the same solvent. The progress of the resolution was tested on small samples from each crystallisation, from which the acid was liberated and measured in acetone solution.

Crystallisation	1	2	3	4
ml methanol	70	85	65	45
g salt obtained	15.0	9.5	6.9	5.3
$[\alpha]_D^{25}$ of the acid	+65.5°	+120.5°	+122°	+123°

The mother liquors from the two first crystallisations were evaporated to dryness and the acid liberated; 11.2 g with $[\alpha]_D^{25} = -59°$ were obtained. This acid (0.06 mole) was dissolved with 7.2 g (0.06 mole) (+)-α-phenyl-ethylamine in 50 ml methanol + 35 ml water. The salt obtained on standing at room temperature for 24 hours was recrystallised and the course of the resolution tested as described above.

Crystallisation	1	2	3
ml methanol	50	50	40
ml water	35	20	20
g salt obtained	11.6	9.4	7.7
$[\alpha]_D^{25}$ of the acid	−117°	−122.5°	−122.5°

(+)-Benznorcaradiene-carboxylic acid. The β-phenyl-*iso*propylamine salt (5.0 g) was decomposed with dilute sulphuric acid and the organic acid extracted with ether. After evaporation of the ether, 3.0 g of crude acid was obtained. It was recrystallised from formic acid, carbon tetrachloride + petroleum ether and once more from formic acid. 2.2 g of pure acid were obtained as fine colourless needles with m.p. 126.5–127.5°. The racemic acid has a quite different crystal habit, forming thin, glistening, rectangular plates or flakes.

0.2622 g acid: 12.41 ml 0.1134 *N* NaOH.
$C_{12}H_{10}O_2$ Equiv. wt. calc. 186.2, found 186.3.
0.1206 g dissolved in acetone to 10.00 ml: $\alpha_D^{25} = +2.925°$ (2 dm).
$[\alpha]_D^{25} = +121.3°$; $[M]_D^{25} = +226°$.

(−)-Benznorcaradiene-carboxylic acid. The α-phenyl-ethylamine salt (7.5 g) was decomposed and the acid recrystallised as described above, in all four times. 3.0 g of pure acid was obtained. M.p. 126.5–127.5°.

0.2725 g acid: 12.90 ml 0.1134 *N* NaOH.
$C_{12}H_{10}O_2$ Equiv. wt. calc. 186.2, found 186.3.

The activity data are given in Table 1.
The activity in acetone solution was also determined at a higher concentration:

0.2057 g dissolved in acetone to 10.00 ml: $\alpha_D^{25} = -4.96°$ (2 dm).
$[\alpha]_D^{25} = -120.6°$; $[M]_D^{25} = -224.5°$.

It is obvious that the rotatory power decreases with increasing concentration. This explains why somewhat higher values were obtained for the maximum activity in the course of the resolution; in these measurements the concentration was rather low.

Table 1. Rotatory power of (−)-benznorcaradiene-carboxylic acid.
g = g acid; ml = ml solution.

	g	ml	α_D^{25} (2 dm)	$[\alpha]_D^{25}$	$[M]_D^{25}$
Water (ion)	0.1164	9.98	−4.58°	−196.3°	−366.5°
Ethanol (abs.)	0.1103	10.00	−3.24°	−146.9°	−273.5°
Acetic acid	0.1118	10.00	−3.12°	−139.5°	−260°
Acetone	0.1073	10.00	−2.61°	−121.6°	−226.5°
Chloroform	0.1055	10.00	−2.40°	−113.7°	−212°
2.2.4-Trimethylpentane	0.1077	10.00	−2.09°	−97.0°	−180.5°
Benzene	0.1118	9.98	−1.83°	−81.7°	−152°

SUMMARY

The benznorcaradiene-carboxylic acid (VI) has been resolved into the optically active forms. The rotatory power found was $[\alpha]_D^{25} = +121.3°$ and −121.6° respectively (in acetone solution).

Preliminary experiments show that both forms are anti-auxins, the (−)-form being more effective.

ACKNOWLEDGEMENTS

The author is indebted to Dr. G. Juppe, Chemical Institute, Munich, for an ample supply of racemic acid and to Dr. J. M. F. Leaper, Ambler, Pennsylvania, for a gift of (−)-β-phenyl-*iso*propylamine. Thanks are also due to Mr. S. Lindenfors for assistance with the irradiation experiments and to Professor B. Åberg, Royal Agricultural College, Uppsala, for kindly reporting the preliminary results on the growth-regulating effect.

Chemical Institute, University of Uppsala, January 1958.

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Tryckt den 3 juni 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Bd 12 nr 47

Vorgelegt am 12. Februar 1958

Neue Amino- und Nitro-Chinolin-Derivate und N-Oxyde

Von Hans von Euler und Hans Hasselquist

In einer vorhergehenden Mitteilung (1) wurden neue Amino- und Amino-chlor-Derivate des *p*- und *o*-Xylols beschrieben. In Fortsetzung der genannten Untersuchung haben wir besonders Amino- und Nitro-Chinolin-Verbindungen und N-Oxyde derselben untersucht, die nach den Ergebnissen von Eiji Ochiai und Mitarb. (2) und von Waro Nakahara, F. Fukuoka und T. Sugimura (3) in mehrfacher Hinsicht grosses Interesse verdienen.

Zur Methodik der N-oxyd-Darstellung

Zur Darstellung von N-Oxyden, besonders solchen, die sich von aromatischen Nitro-Verbindungen ableiten, haben E. Ochiai und Okamoto (3), denen man besonders die neueren Ergebnisse über N-Oxyde verdankt, Hydroperoxyd verwendet.

Analoge Oxyde von Chinolin, Ischinolin, Chinaldin und Pyridin wurden vor längerer Zeit von J. Meisenheimer und E. Stotz (4) unter Verwendung von Benzopersäure synthetisiert. Wir haben ein ähnliches Verfahren benutzt und zu diesem Zweck die Benzopersäure-Lösung in Chloroform, nach Pummerer (5) Benzoylperoxyd in Toluol mit Alkohol und Natrium versetzt.

1 g 6-Nitro-Chinolin liess man mit einem Überschuss von Benzopersäure 24 Stunden bei Zimmertemperatur stehen, worauf die Lösung, die sich nun intensiv gelb färbte, mit einigen Tropfen konc. Salzsäure versetzt wurde. Die Chinolinverbindung ging dabei in die HCl-Schicht über unter Bildung des farblosen salzsauren Salzes.

Die HCl-Schicht wurde im Vacuum bei Zimmertemperatur zur Trockne verdampft, wobei ein Teil des salzsauren Salzes HCl abgibt und sich dadurch wieder gelb färbt.

Das Produkt wurde zweimal aus Methanol umkristallisiert und dadurch in Form kleiner, gut ausgebildeter intensiv gelber Prismen vom Schmpt. 216° erhalten. Dieselben erwiesen sich als vollständig Cl-frei.

Analyse (Sbst. 272)

Ber. für $C_9H_6N_2O_3$:	C 56,85 %	H 3,18 %	N 14,73 %
Gefunden:	56,80	3,49	14,64

Das Produkt 272 erwies sich also als 6-Nitro-chinolin-N-oxyd.

Darstellung des 3-Amino-5-nitro-1,2-dimethylbenzols

Die Synthese wurde im Wesentlichen nach der Methode von E. Noelting u. Mitarb. (6) folgendermassen ausgeführt:

17,2 ml 3-Amino-1,2-dimethylbenzol wurden unter Umrühren und Abkühlung in 200 ml konc. Schwefelsäure eingetragen. Nach vollständiger Auflösung wurde auf –15° abgekühlt, worauf man tropfenweise eine auf die gleiche Temp. abgekühlte Lösung von 7,2 ml konc. Salpetersäure (1,42) in 22 ml konc. Schwefelsäure zugab. Die Temperatur durfte dabei –10° nicht übersteigen. Nach dem Zusatz liess man die Reaktionsmischung 30 Min. bei der gleichen Temp. stehen und goss sie dann auf 500 g Eis.

Das Sulfat des 3-Amino-5-nitro-1,2-dimethylbenzols fiel dabei in Form farbloser Kristalle aus, die nach Abfiltrieren mit wenig Aceton gewaschen wurden. Ausbeute 12,5 g (58 % der Theor.). Das Sulfat war praktisch frei von Isomeren und konnte direkt zur Synthese der Chinolin-Verbindung (Sbst. 273) verwendet werden. Durch Verdünnung des Filtrates vom abfiltrierten Sulfat mit 750 ml Wasser erhielten wir eine gelbe bis gelbrote kristallinische Fällung von 3-Amino-4-nitro-1,2-dimethylbenzol; durch Zusatz von wenig Ammoniak kristallisierte das 3-Amino-6-Nitro-1,2-dimethylbenzol in kleinen braunen Prismen aus (Sbst. 273).

Umsetzung von 3-Amino-5-nitro-dimethylbenzol mit Glycerin

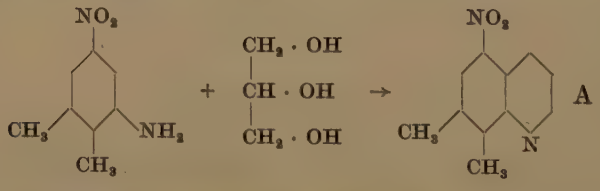
5 g 3-Amino-5-nitro-1,2-dimethylbenzol-sulfat wurden mit 2,1 g As_2O_5 und ferner mit 5,5 g Glycerin und 1,5 ml konc. Schwefelsäure mit Rückflusskühler unter Schütteln erhitzt bis eine homogene Schmelze entstand. Durch 1-stündiges gelindes Erhitzen erhielt man eine braunrote Lösung. Dieselbe wurde nun mit 150 ml Wasser verdünnt und vorsichtig mit starker Natronlauge bis pH 4 versetzt. Das hierbei gebildete Chinolin fiel in Form eines mikrokristallinischen braunen Pulvers aus, das mit Wasser gründlich gewaschen wurde. Ausbeute an Rohprodukt: 2,6 g vom Schmpt. 108–114°.

Das Rohprodukt wurde in ein wenig Benzol gelöst, mit aktiver Kohle vermischt, wodurch eine fast farblose Lösung entstand, die auf ein kleines Volumen eingedunstet und mit etwas Petroleumäther versetzt wurde. Die Substanz wurde nun in Form langer, schwach gefärbter Stäbchen vom Schmpt. 119° erhalten. Weiteres Umkristallisieren erhöhte den Schmelzpunkt nicht.

Analyse (Subst. 273)			
Ber. für $C_{11}H_{10}O_2N_2$:	C 65,33 %	H 4,99 %	N 13,86 %
Gefunden:	65,29	5,07	13,34

Die Sbst. 273 ist äusserst leicht löslich in Benzol und Chloroform, bedeutend schwerer löslich in Methanol, Äthanol und Aceton, schwerlöslich in Wasser.

Wir nehmen für diese bisher nicht bekannte Substanz die folgende Struktur A an:



Reduktion des 7,8-dimethyl-5-nitro-chinolins (Sbst. 273)

7 g $SnCl_2 \cdot 2H_2O$ wurden in 10 ml konc. HCl gelöst und mit 1 g der Sbst. 273 unter Umrühren versetzt, wobei Wärmeentwicklung beobachtet wurde. Nach

60 Min. langem Verweilen auf dem kochenden Wasserbad kristallisierte ein Zinnsalz der reduzierten Verbindung in Form grosser brauner Prismen aus. Das Ganze wurde im Vacuum zur Trockne verdampft und hierauf mit 100 ml Wasser versetzt. Dabei gingen die braunen Prismen in ein intensiv rotbraunes Kristallpulver über, das sich allmählich auflöste. Nun wurde das Ganze mit so viel 20 %iger Natronlauge versetzt, dass das zunächst ausfallende Stanni-Hydrat in Lösung ging. Ein ungelöster Rest eines grau-grünen mikrokristallinischen Pulvers wurde abfiltriert, mit Wasser gewaschen und getrocknet. Ausbeute an Rohprodukt 0,82 g vom Schmpt. 134–136°.

Beim Behandeln des Rohproduktes mit Methanol verblieb ein amorpher Anteil ungelöst und wurde abfiltriert. Das Ganze wurde im Vacuum zur Trockne verdampft. Beim Umkristallisieren der Substanz aus Benzol wurde dieselbe in Form schwach gelber Nadeln mit hellgrüner Fluorescenz erhalten. Ob diese von der Substanz selbst herrührt oder von einer Spur von Beimengung konnte noch nicht festgestellt wurden. Schmpt. 140–141°.

Analyse (Sbst. 274)

Berechnet für $C_{11}H_{12}N_2$:	C 76,71 %	H 7,02 %	N 16,27 %
Gefunden:	77,00	7,27	15,91

5-Amino-7,8-dimethyl-chinolin

Die Substanz 274 ist äusserst leichtlöslich in Methanol, Äthanol und Aceton, etwas schwerer löslich in Benzol, schwerlöslich in Wasser und in Alkalilauge, löslich durch Salzbildung mit Säuren.

Bei 760 mm Hg kann die Sbst. 274 ohne erhebliche Zersetzung destilliert werden.

Acetylierung der Substanz 274

200 mg der Sbst. 274 wurden mit 2 ml Essigsäure-anhydrid 5 Minuten gekocht, wodurch die Sbst. in Lösung ging. Beim Erkalten kristallisierte die Acetylverbindung in Form langer farbloser Nadeln aus. Das unveränderte Essigsäure-anhydrid und gebildete Essigsäure wurden im Vacuum vollständig entfernt. Die zurückbleibende trockene Kristallmasse wurde hierauf aus Benzol umkristallisiert, wodurch die Acetylverbindung als farblose verfilzte Nadeln vom Schmpt. 203–205° erhalten wurde. Ausbeute fast quantitativ.

Bei weiterem Umkristallisieren aus Benzol wurde der konstante Schmpt. 209–209,5° erreicht.

Analyse (Subst. 276)

Berechnet für $C_{13}H_{14}ON_2$:	N 13,08 %
Gefunden:	12,16

Oxydation der Sbst. 274 (7,8-Dimethyl-5-nitro-chinolin) mit H_2O_2

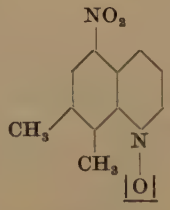
0,5 g der Sbst. 273 in 10 ml Eisessig gelöst, wurden mit 1,5 ml 30 %igem Perhydrol auf dem Wasserbad bei 70° zwei Stunden lang erwärmt. (Kürzere Reaktionszeit liefert schlechtere Ausbeuten, ebenso längere Reaktionszeit, da die Substanz dann amorphe und schmierige Oxydationsprodukte liefert. Mit Benzopersäure wird in diesem Fall nur die sehr geringe Ausbeute von etwa 10 mg aus 0,5 g Ausgangsmaterial erhalten.) Nach Beendigung der Reaktionszeit wurde die kräftig gelbbraune Lösung im Vacuum bei 50° eingeengt. Der Rückstand, eine gelbbraune, teilweise kristallinische schmierige Masse, enthielt noch etwas Essigsäure sowie

Hydroperoxyd. (Eindunsten bis zur Trockne erniedrigt die Ausbeute mit ca. 90 %, da das Peroxyd amorphe Produkte hervorruft.)

Die schmierige Masse wurde bei Zimmertemperatur wiederholt mit Chloroform extrahiert und die gesammelten Chloroformextrakte wurden nach Entwässerung mit Natriumsulfat und Filtration im Vacuum vollständig eingedunstet: die rückständige, stark gelbe Kristallmasse enthielt noch unverändertes Ausgangsmaterial, das in Chloroform äusserst leichtlöslich ist. Bei Zusatz von 3 ml Chloroform in der Kälte verblieb das gebildete N-Oxyd zum grössten Teil ungelöst in Form eines intensiv gelben Kristallpulvers, das abfiltriert werden konnte. Das Rohprodukt, 85 mg, schmolz bei 183–185° unter Braunfärbung und Zersetzung. Durch zweimaliges Umkristallisieren aus wenig Chloroform wurde die Substanz 275 in Form kleiner, stark gelber Stäbchen erhalten, die bei 186–187° unter Zersetzung schmolzen.

Analyse (Subst.) 275			
Berechnet für $C_{11}H_{10}O_3N_2$:	C 60,54 %	H 4,62 %	N 12,84 %
Gefunden:	59,65	4,74	13,11

Wir nehmen für die Sbst. 275 die Struktur B an.



B

5-Nitro-7,8-dimethyl-chinolin-N-oxyd

Sbst. 275 ist leicht löslich in Methanol und Aceton; ziemlich leicht löslich in Chloroform und Benzol; unlöslich in Wasser und in Petroleumäther.

Von unseren Versuchen an N-Oxyden teilen wir hier nur unsere Erfahrungen an einigen Nitrochinolinen mit. Weitere Beobachtungen werden an anderer Stelle unter Besprechung der durch die N-Oxyd-Gruppe eintretenden Mesomerie-Effekte beschrieben.

4-Nitrochinolin-N-oxyd, dargestellt von E. Ochiai u. Mitarb. (2), besitzt nach Nakahara, Fukuoka und Sugimura (3) eine kräftige *cancerogene Wirkung*. Dieser Nachweis ist um so auffallender, als manche Chinone und Chinolin-Derivate eine deutliche *krebshemmende* Wirkung zeigen und da nach Nakamura und Mitarb. 4-Nitrochinolin-N-oxyd bei Konzentrationen von 0,002 % Mäuse-Sarkom-Zellen in vitro tötet und in vivo bei Tieren mit Ehrlich-Ascites-Carcinomen die Lebensdauer auffallend verlängert. Es zeigt sich also bei 4-Nitrochinolin-N-oxyd die gleiche Erscheinung, die Haddow und Sexton (7) an Urethanen und K. H. Bauer (Das Krebsproblem) an cancerogenen Kohlenwasserstoffen beobachtet hatten.

Behandelt man die Haut normaler Mäuse dreimal wöchentlich mit 0,25%iger Benzollösung, so entstehen nach Nakahara Papillome in einer Periode zwischen dem 80. und 140. Tag in allen Versuchstieren und maligne Tumoren zwischen dem 120. und 200. Tag. Am 150. Tag trugen 100 % der Mäuse Tumoren und in 36 % dieser Fälle waren diese Tumoren bereits bösartig. Metastasen wurden

nur in wenigen Fällen gefunden. Einer dieser Tumoren konnte mit Erfolg auf normale Mäuse transplantiert werden.

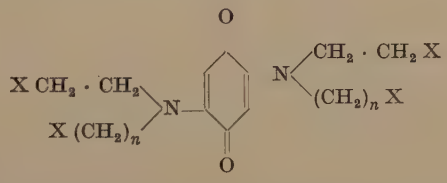
4-Nitrochinolin-N-Oxyd ist nach NAKAHARA als erste bicyclische Verbindung, anzusehen, deren carcinogene Wirkung derjenigen der kräftigsten polycyclischen Kohlenwasserstoffe nahekommt.

Das bemerkenswerteste N-Oxyd ist das von YOSHIDA und ISHIDATE (8) studierte N-oxyd-Lost, das bis jetzt die wirksamste krebsheilende Substanz sein dürfte und als „Nitromin" von YOSHIDA auch klinisch mit Erfolg verwendet wurde. Die Ergebnisse von YOSHIDA wurden in Deutschland durch DRUCKREY (9) und BROCK (10) am *Mitomen* der Asta-Werke voll bestätigt (10 b).

Nach ISHIDATE ist das Lost-N-oxyd eine inaktive Transportform im Sinne von DRUCKREY (15). Eine chemotherapeutisch sehr geeignete Transportform, ein N-Lost-Phosphamid, B 518, Asta, wurde von N. BROCK (10) beschrieben.

Die Umwandlung des Nitromins im Tierkörper und das Auftreten von Zwischenprodukten bleiben noch weiter aufzuklären.

Bezüglich der Stickstoff-Chinone verweisen wir auf die interessante Untersuchung von W. GAUSS und S. PETERSEN (11) sowie die Übersicht von G. DOMAGK, ferner auf eine vorläufige Mitteilung aus diesem Institut von STIG ÅKERFELDT (Arkiv Kemi 7 nr 38, 1954) und auf die Arbeiten von H. HOLZER (12) und K. H. KÖNIG (13) über den folgenden Typus:



Siehe auch EULER u. HASSELQUIST (14) SCHMÄHL u. DRUCKREY (15).

Stockholm, Inst. für organ. chem. Forschung.

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Tryckt den 3 juni 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 48

Vorgelegt am 12. März 1958

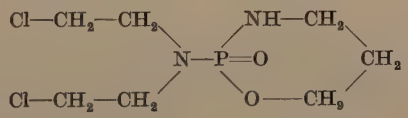
Zur Chemie der N-Oxyde

Von Hans von Euler und Hans Hasselquist

Nach dem Befund von Ishidate und Yoshida (1) erfährt die anticanceröse Wirksamkeit des N-Lostes durch dessen Oxydation zu N-Oxyd-Lost (2) eine wesentliche Verstärkung; dabei ist die Toxicität des N-Oxyd Lostes und der von Ishidate und Sakurai (3) dargestellten Derivate desselben geringer als diejenige der entsprechenden Amine. In Bestätigung der japanischen Ergebnisse konnte Druckrey (4) feststellen, „dass durch das N-Oxyd-Lost sich sowohl am Yoshida-Ashites-Sarkom als auch am Jensen-Sarkom und am Walker-Tumor eine sichere und endgültige Heilung aller Tiere erzielen lässt, wenn die Behandlung in den ersten Tagen nach der Impfung erfolgt, ohne dass auch nur ein Tier durch die Giftwirkung verloren ging". Ferner zeigten Untersuchungen von Druckrey, dass das N-Oxyd-Lost eine Transportform darstellt, die durch den Erreger, vornehmlich die Krebszelle, in die hochaktive „Wirkform" übergeführt wird. Für die Transportform ist, wie Brock betont, nicht nur eine weitgehende Unwirksamkeit sondern auch der Nachweis einer schnellen Resorption und Entgiftung bedeutsam.

Die Darstellung des N-Oxyd-Lostes durch Oxydation des N-Lostes mit Peroxyden war wohl die letzte grössere Arbeit Max Bergmanns (2).

Eine wirksame Transportform, der cyklische N-Lost Phosphamidester B 518, wurde in den Asta-Werken (N. Brock) von Arnold und Bourseaux synthetisiert, und wird nun von den Asta-Werken (Brackwede, Westf.) dargestellt. (Brock, Arzneimittelforsch. *8*, 1 (1958).)



B 518

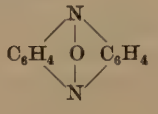
Ishidate hat angenommen, dass unter Abspaltung eines Chlorions und Einbeziehung des Aminoxyd-Sauerstoffs in einem Prozess A eine Zwischenform und Detoxication auftritt, während in einem Prozess B eine biologisch aktive Form (HN2) entsteht.

Bei den N-Losten wird das Cl-Atom sehr leicht unter Bildung von Radikalen am Kohlenstoffatom abgespalten, was die biologische Wirkung wesentlich beeinflusst. Preussmann (5) hat in Laboratorium von Druckrey die Chlor-Abspaltbarkeit von Acyl-Derivaten des Bis-(β-Chloräthyl)-amins untersucht und ist zu folgendem Ergebnis gekommen: Die untersuchten Acyl-Verbindungen zeigten keine oder nur äusserst schwache Chlorid-Abspaltung und erwiesen sich an transplantierten

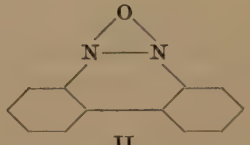
Rattentumoren als nicht-Tumor-hemmend. Preussmann zieht daraus den Schluss, dass die chemotherapeutische Wirksamkeit der bis-β-Chloräthylamine ein Optimum haben muss.

Die cancerogene Wirkung von N-oxyd-Lost auf Ratten wurde von D. STEINHOFF und BOK TSCHIN KUK untersucht. Sie ist sicher ausserordentlich viel schwächer als beim einfachen N-Lost. Nach STEINHOFF u. B. T. KUK muss das N-oxyd-Lost als ein cancerogener Stoff angesehen werden, dessen carcinogene Dose allerdings etwa 200mal so hoch sein muss als die therapeutische (Z. Krebsf. *62*).

Ältere Angaben über N-Oxyde: Als N-Oxyde bezeichnete Stoffe wurden durch Einwirkung von Nitrobenzol und trockenem Ätznatron auf Anilin bzw. β-Naphthylamin dargestellt. WOHL und AUE (6a) schrieben ihren Produkten, dem Phenazin-N-Oxyd bzw. dem Naphthophenazin-N-Oxyd Strukturen vom Typus I zu. Gegen diese Formeln können aber vom Standpunkt der Atomtheorie Einwände erhoben werden.



I



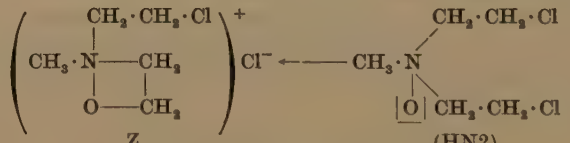
II

Inwieweit das Formelbild I zutrifft wird sich entscheiden lassen, wenn der eine der beiden Benzolringe einen Substituenten enthält; man hat dann zu untersuchen, ob das Produkt in *zwei* isomeren Formen auftritt. Das Gleiche gilt bez. II.

ULLMANN und DIETERLE (6b) formulierten ihr Diphen-azo-N-Oxyd nach II.

Schon vorher hatten BAMBERGER und TSCHIRNER (7) das N,N′-Dimethyl-anilin-Oxyd durch Oxydation von Dimethylanilin dargestellt und eingehend studiert.

Auch nach der Auffassung von DRUCKREY muss aus dem N-Oxyd-Lost in vivo ein anderes biologisch aktives Produkt, vermutlich Zwischenprodukt, entstehen als das N-Lost.



Z (HN2)

Die aktive Form von HN2 bleibt noch aufzuklären.

Zunächst haben wir uns gefragt, ob die Bindung des Sauerstoffs im Lost-N-oxyd derjenigen im Azoxybenzol entspricht, für die nach A. ANGELI die asymmetrische Form $C_6H_4\text{–}\underset{|\underline{O}|}{N} = N\text{–}C_6H_4$ angenommen wird, die ja auch mit der PAULINGschen Oktettregel in Übereinstimmung steht (8).

1 mmol Ascorbinsäure in 2 ml Wasser wurde mit einer Lösung von 0,5 mmol Azoxybenzol in 3 ml Methanol 4 Stunden bei 20° stehen gelassen. Keine Reaktion konnte beobachtet werden. Nach Verdünnung mit wenig Wasser wurde mit Petroleumäther extrahiert. Nach Abdunsten des Petroleumäthers wurde das Azoxybenzol unverändert zurückerhalten. Es war also keine Reduktion eingetreten.

Nitroso-dimethylanilin. Nach Mishung vom 1 mmol Ascorbinäsure mit 0,5 mmol Nitroso-dimethyl-anilin in 3 ml Methanol tritt sofort Reaktion ein, und nach etwa 1 Minute war die an-

fangs grüne Lösung gelblich. Nach Zusatz von etwas NaAc wurde mit Äther extrahiert. Nach Eindunsten des getrockneten Äthers hinterblieb ein schwach gefärbtes Öl, das auf dem siedenden Wasserbad acetyliert wurde. Als Produkt erhielten wir farblose lange Nadeln, die sich mit *p*-Acetamino-dimethylanilin, Schmpt. 131° identisch erwiesen. Nitroso-dimethylanilin wird also in saurer Lösung von Ascorbinsäure zu *p*-Amino-dimethylanilin reduziert.

In ganz analoger Weise wie Nitroso-dimethylanilin wurde 6-Nitro-chinolin-N-Oxyd in Methanol behandelt, mit welchem nach 4 Stunden *keine Reaktion* eintrat.

Wie die obigen Versuche zeigen, besteht hinsichtlich der Reduzierbarkeit durch Ascorbinsäure ein *erheblicher Unterschied* zwischen der Nitrosogruppe des Nitroso-dimethylanilins einerseits und der NO-Gruppe des Nitro-chinolin-N-Oxyds und des Azoxybenzols andererseits. Dieser Unterschied muss in den Strukturformeln der drei verschiedenen Stofftypen zum Ausdruck kommen.

Für die Sauerstoff-Bindung in der Nitrosogruppe, die durch eine Doppelbindung dargestellt wird, sind 2 Elektronenpaare anzunehmen, während die Sauerstoff-Bindung in den N-Oxyden nur durch *ein* Elektronenpaar vermittelt sein dürfte und nach der älteren Bezeichnungsweise durch *einen* Valenz-Strich dargestellt wird.

Die experimentell feststellbare Eigenschaft, die zur Kennzeichnung der NO-Gruppe bzw. ihrer Träger dient, ist ihr *basischer* bzw. *saurer Charakter*, gemessen durch den Grad ihrer Salzbildung mit Säuren bzw. Basen, und die daraus zu berechnende Dissociationskonstante. Azoxybenzol geht überwiegend durch Säuren in Lösung. Wie MEISENHEIMER (Ber. *56*, 1353 (1923)) betont, kann ein dreiwertiges N-Atom nicht die Bildung eines asymmetrischen Moleküls veranlassen.

Bald darauf hat H. PHILLIPS (9) ähnliche Betrachtungen angestellt über die Beziehung der Asymmetrie eines (S-)Atoms und der Bindung an 3 verschiedene Gruppen.

Am Beispiel der *Aminoxyde*, welche eine Stickstoff-Sauerstoff-Doppelbindung enthalten, hat MEISENHEIMER (10) im Anschluss an VAN'T HOFF (11) das Auftreten von asymmetrischen Formen besprochen.

Chinolin-N-Oxyde

Die anti-canceröse Wirkung der von E. OCHIAI und Mitarb. (2) synthetisierten Chinolin-N-Oxyde wurde von FUKUOKA untersucht. Durch Konzentrationen von 0,002 % wurden Mäuse-Sarkom-Zellen in vitro vollständig getötet (12). In-vivo-Versuche an Ehrlich-Ascites-Carcinom-Mäusen ergaben, wenn die Behandlung mit 7 mg per kg per Tag 24 Stunden nach der Injektion begann, eine vollständige Hemmung (Heilung) des Ascites-Tumors.

Statt des in Wasser sehr wenig löslichen 4-Nitro-chinolin-N-oxydes verwendete FUKUOKA auch das wasserlösliche Kaliumsalz des 4-Nitro-Carboxychinolin-N-Oxydes, das weniger toxisch ist als die wasserunlöslichen Chinolin-N-Oxyde. Im in-vivo-Versuch erwies sich die Überlebenszeit an Ehrlich-Carcinom-Mäusen, die mit 125 mg per kg-Dosen an 7 aufeinander folgenden Tagen behandelt wurden, 36 Tage gegenüber 11 Tagen der unbehandelten Tiere.

Die verschiedene Empfänglichkeit von Ratten-Stämmen gegen Stickstoff-Lost in Zusammenhang mit Ungleichheiten des Stoffwechsels wurde von Y. MIURA (13) untersucht. Ob diese Verschiedenheit sich sowohl bez. der heilenden wie bez. der cancerogenen Wirkung geltend macht, bleibt noch festzustellen.

Zu erwähnen ist die Beobachtung von FUKUOKA, dass auch 4-Nitro-chinolin-N-

oxyde wie N-Lost eine stark *cancerogene* Wirkung ausüben. Durch wöchentlich 3malige Behandlung von Mäuse-Haut wurden in allen Tieren innerhalb 80–140 Tagen Papillome erzeugt. Die so hervorgerufenen malignen Tumoren waren histologisch schuppenartige Zellen-Carcinome oder Fibrosarcome.

Versuche über Nitro-chinolin-Derivate und deren N-Oxyde

6-Nitro-chinolin- und Benzoepersäure

Darstellung durch Einwirkung von überschüssiger Benzoepersäure (dargestellt nach PUMMERER u. Mitarb. (14)) in Chloroform. Gelbe Prismen vom Schmpt. 215–216°.

Analyse. Sbst. 272. Ber. für $C_9H_6N_2O_3$	C 56,85 %	H 3,18 %	N 14,73 %	
Gef.	56,80	3,41	14,64	

Das Produkt 272 erwies sich also als 6-Nitro-chinolin-N-oxyd.

Analoge Derivate von Isochinolin, Chinaldin und Pyridin sind von J. MEISENHEIMER und E. SCHOLTZ (15) sowie von J. MEISENHEIMER (16), in neuerer Zeit von E. OCHIAI u. T. OKOMOTO (17) dargestellt worden.

7,8-Dimethyl-5-nitro-chinolin, $C_{11}H_{10}O_2N_2$, Sbst. 273 Wurde neu dargestellt aus 3-Amino-5-nitro-dimethyl-benzol und Glycerin (EULER u. HASSELQUIST (18)).

Die Sbst. 273 wurde mit $SnCl_2$ in salzsaurer Lösung reduziert zu dem bisher unbekannten 7,8-Dimethyl-5-Amino-chinolin, $C_{11}H_{12}N_2$ (Sbst. 274).

Darstellung von 5,6-Dimethyl-chinoxalin

Die Suspension von 1,5 g 3,4-Diamino-1,2-dimethyl-benzol wurde mit 2,3 g Glyoxal-bisulphit in 25 ml Wasser 4 Stunden auf 65° erwärmt. Nach Ablauf der Reaktion wurde das braunrote Öl mit Wasser versetzt und im Scheidetrichter wiederholt mit Petroleumäther ausgeschüttelt. Die gesammelten Extrakte wurden mit Natriumsulfat getrocknet, zu einem kleinen Volumen eingedunstet und mit Eiswasser gekühlt, wodurch sich ev. noch anwesendes freies Diamin entfernen lässt. Die Petroleumäther-Lösung wurde nun mit konz. Salzsäure ausgeschüttelt, wobei das in der Petroleumäther-Schicht anwesende Dimethyl-chinoxalin als HCl-Salz in die wässrige Lösung überging. Durch Eindunsten dieser Schicht zur Trockene wurde ein hell gelbbraunes Kristallpulver erhalten, das nach dem Umkristallisieren bei 177–178° unter Zersetzung schmolz. Ausbeute fast quantitativ. Sbst. 277.

Analyse. Berechnet für $C_{10}H_{10}N_2 \cdot HCl$	C 61,70 %	H 5,18 %	N 14,39 %	Cl 18,21 %	
Gef.	61,41	H 5,14	N 14,20		

Das salzsaure Salz 277 ist leicht löslich in Wasser und Methanol, ziemlich leicht löslich in Äthanol und in Aceton.

Die freie Chinoxalin-Base ist ein Öl, das bisher nicht zur Kristallisation gebracht werden konnte.

Im Gersten-Keimtest erwies sich das Salz 277 (auf pH 6,7 gebracht) in der Konzentration von 10 mg in 10 ml Wasser (16 st. Quellen in Wasser bei 18°, dann Quellen in der Konzentration 10 mg in 10 ml Wasser) als nicht toxisch; die Keimungsgeschwindigkeit sowie die Länge der Keimblätter und Wurzeln nach 6 Tagen war unverändert gegenüber Wasser.

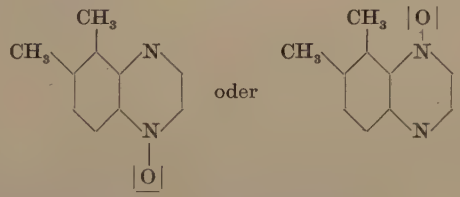
Darstellung von 5,6-Dimethyl-chinoxalin-N-oxyd

Aus 1 g 5,6-Dimethyl-chinoxalin-HCl (Sbst. 277) in 10 ml Wasser gelöst, wurde mit Überschuss von NaAc die Base freigemacht, die sich mit Petroleumäther leicht extrahieren liess. Die durch Eindunsten des Extraktes erhaltene freie Base wurde in 8 ml Eisessig gelöst und mit 4 ml einer 30%igen Hydroperoxydlösung 10 Stunden bei 60° stehen gelassen. Aus der nun stark gelben Lösung wurde durch Zusatz von Wasser das Oxydationsprodukt in Form hellgelber Krystalle ausgefällt. Um die Fällung vollständiger zu machen, wurde die Lösung mit NaOH auf pH = 6,5 neutralisiert, worauf das ganze Oxydationsprodukt dreimal mit Chloroform extrahiert wurde. Aus den gesammelten getrockneten und eingedunsteten Chloroformschichten wurde eine Kristallmasse erhalten, die durch Umkristallisieren aus warmem Benzol im Form grosser hellgelber Blättchen vom Schmpt. 152–153° erhalten wurde. Sbst. 278.

Analyse. Ber. für Mono-Oxyden $C_{10}H_{10}ON_2$:	C 68,94 %,	H 5,79 %,	N 16,08 %
Gef.	68,19	5,67	15,72
Ber. für Di-Oxyden $C_{10}H_{10}O_2N_2$:	63.14	5,30	14,73

Die Analyse zeigt also Übereinstimmung mit $C_{10}H_{10}ON_2$, dem Mono-N-oxyd der Sbst. 277.

Die Sbst. 278 ist leichtlöslich in Chloroform, ziemlich leicht löslich in warmem Benzol, schwerlöslich in Wasser.



Im Gersten-Keimungs-Test erwies sich das N-Oxyd 278 in der Koncentration von 10 mg per 100 ml Wasser (pH = 6.7) als nicht toxisch; ein *ganz geringe* Beschleunigung der Keimungs-Geschwindigkeit konnte in einigen Versuchen beobachtet werden.

Das Auftreten eines bis-N-oxydes eines Dihydroxyphenazins als Produkt von Chromobacterium iodinum (Iodinin) wurde von McIlvain vermutet.

Oxydation des Nikotinsäureamids mit Hydroperoxyd

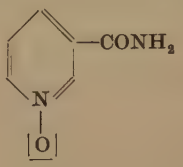
1,6 g Nikotinsäureamid in 8 ml Eisessig gelöst, wurden mit 4 ml einer 30%igen Hydroperoxydlösung 48 Stunden bei 65° stehen gelassen. Dann wurde die ganze Lösung im Vakuum bei 80° bis auf ein kleines Volumen eingeengt, wobei farblose, beinahe verfilzte Nadeln ausfielen. Als der Rückstand nur mehr 3–4 ml betrug wurde das Einengen unterbrochen, die ausgeschiedenen Kristalle wurden abfiltriert und mit wenig kaltem Wasser gewaschen. Rohprodukt 1 g vom Schmpt. 250–262°.

Aus dem Filtrat fielen weitere 0,3 g Rohprodukt aus. Durch Umkristallisieren wurde das Produkt praktisch rein erhalten; Schmpt. 287–288°.

Ausgangsmaterial, Nikotinsäureamid Schmpt. 131°, Nikotinsäure Schmpt. 228°. Die Sbst. 279 ist, zum Unterschied von unseren früher dargestellten N-Oxyden farblos.

Analyse. Berechnet für $C_6H_6O_2N_2$: C 52,13,	H 4,38,	N 20,27
Gef.	52,15	4,66	20,04

Die Sbst. 279 ist ziemlich löslich in kaltem Wasser und zwar löslicher als das Nikotinsäureamid selbst; wenig löslich in Chloroform, ziemlich schwerlöslich in Methanol, während sich das Nikotinsäureamid darin äusserst leicht löst.



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Tryckt den 3 juni 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 49

Communicated 26 February 1958 by Arne Fredga

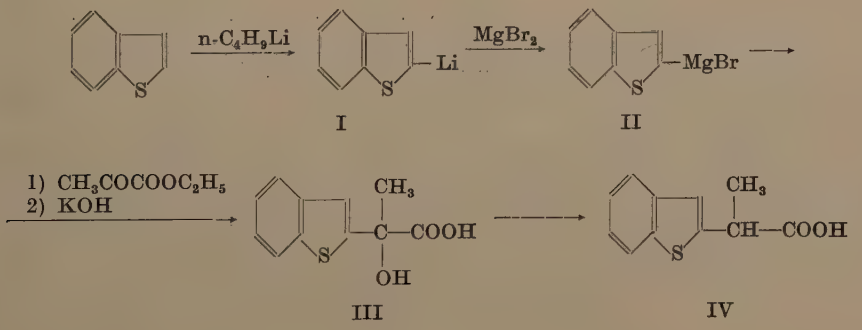
Stereochemical studies. IV

The enantiomers of α-(2-thianaphthenyl)-propionic acid

By Berndt Sjöberg

In connection with the stereochemical studies at this institute on synthetic plant growth regulators, the present author has investigated some aralkyl carboxylic acids. It has been shown that there are interesting stereochemical and also plant physiological differences between the isomeric α-(1-naphthyl)- and α-(2-naphthyl)-propionic acid. In a previous paper the enantiomers of α-(3-thianaphthenyl)-propionic acid have been described (1). From stereochemical as well as from plant physiological points of view it was desirable to extend these investigations to α-(2-thianaphthenyl)-propionic acid (IV).

The racemic acid was prepared in a similar way as the 3-isomer (1). 2-Bromothianaphthene cannot be prepared by direct bromination of thianaphthene. It can be obtained via 2-thianaphthenyllithium (I). However, in this investigation 2-thianaphthenyllithium was directly converted to the Grignard compound (II) by adding magnesium bromide etherate. The Grignard reagent was then reacted with ethyl pyruvate and after saponification α-hydroxy-α-(2-thianaphthenyl)-propionic acid (III) was obtained. This acid was reduced with stannous chloride–hydrochloric acid, with a small amount of hydriodic acid present, to α-(2-thianaphthenyl)-propionic acid (IV).



Several attempts were made to react the lithium compound (I) at low temperature (down to –60°) with ethyl pyruvate. It was not possible in these experiments to get a selective reaction with the keto group, as the lithium compound also reacted with the ester group.

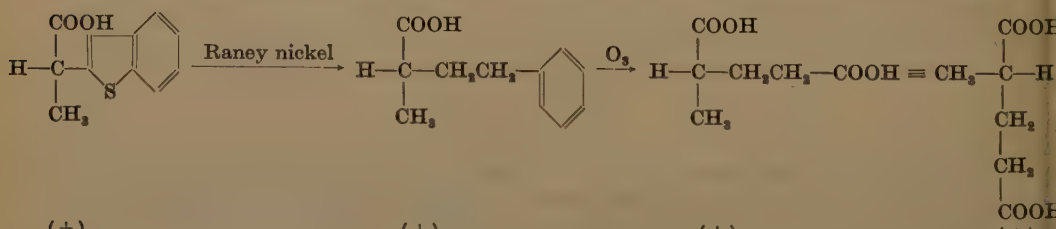
In preliminary experiments on the resolution of α-(2-thianaphthenyl)-propionic acid it was found that the (+)-form could be obtained from morphine, cinchonidine and (−)-α-(2-naphthyl)-ethylamine, while (+)-α-(2-naphthyl)-ethylamine was the only one of the bases tried which gave the (−)-form in a satisfactory way. The best results were obtained with morphine and α-(2-naphthyl)-ethylamine in ethyl acetate but the solubility was too small to use this solvent for larger quantities and so the resolution was carried out using morphine and (+)-α-(2-naphthyl)-ethylamine in dilute ethanol.

In 1953 Fredga described an elegant method for the correlation of optically active aromatic and aliphatic compounds (2). The phenyl compound was connected to a similar thiophene compound by the formation of a quasi-racemate and the thiophene nucleus was desulphurized with Raney nickel forming the corresponding aliphatic compound. Some thianaphthene substituted carboxylic acids have been desulphurized in the same way to the corresponding phenyl substituted acids (3). So it seemed to be a useful method of establishing the configuration of α-(2-thianaphthenyl)-propionic acid, to convert the active acid to α-methyl-γ-phenylbutyric acid (V). The latter acid has been resolved by Levene and Marker (4).

Dextrorotatory α-(2-thianaphthenyl)-propionic acid, $[\alpha]_D^{25} = +31.2°$ in absolute ethanol, was desulphurized with Raney nickel. The isolated acid was analysed and showed to contain practically no sulphur. It was dextrorotatory and had an activity $[\alpha]_D^{25} = +23.3°$ in absolute ethanol and $[\alpha]_D^{25} = +26°$ in homogeneous state. Levene and Marker reported $[\alpha]_D^{32} = 27.1°$ for the homogeneous α-methyl-γ-phenylbutyric acid. They had no determinations in solution.

Levene and Harris assumed that α-methyl-γ-phenylbutyric acid and hydratropic acid with the same direction of rotation also had the same configuration (5). There is very strong evidence that the dextrorotatory hydratropic acid belongs to the D-glyceraldehyde system (6, 7, 8). Thus it seemed to be probable that (+)-α-(2-thianaphthenyl)-propionic acid has the configuration represented by stereoformula IVa. To get a definite proof for the steric relations, the acid V was ozonized into α-methylglutaric acid (VI). The oxalic acid, formed at the ozonolysis, was decomposed by permanganate and the α-methylglutaric acid was easily isolated. The acid was dextrorotatory with $[\alpha]_D^{25} = +23.3°$ (chloroform); m.p. 71–81°. Pure (+)-α-methylglutaric acid has a specific rotation in chloroform of +25.1° and a melting point of 82.5–83° (9). However, the infrared spectra was identical with that of authentic (+)-α-methylglutaric acid. From the melting point curve of the antipodes of α-methylglutaric acid it can be seen that a wide melting point interval can be expected for the partly racemized acid (10).

The configuration of α-methylglutaric acid has been established by Fredga (9) and it has been shown that the (+)-antipode has the configuration VIa which is



(+) IVa (+) V (+) VI (+) VIa

identical with VI. Consequently α-(2-thianaphthenyl)-propionic acid and α-methyl-γ-phenylbutyric acid with the same direction of rotation (mesured in ethanol) also have the same configuration and the dextrorotatory forms are represented by the stereoformulas IVa and V respectively.

The plant growth regulating activity of the enantiomers of α-(2-thianaphthenyl)-propionic acid has been studied by Professor B. Åberg and the results will be published elsewhere (11).

Experimental

All reactions with lithium and magnesium were carried out in ordinary Grignard apparatus in a nitrogen atmosphere.

n-Butyllithium

An ether solution of *n*-butyllithium was prepared according to Wittig (12).

2-Thianaphthenyllithium (I)

This compound was prepared according to the method of Shirley and Cameron (13). A solution of 250 ml of 1-molar *n*-butyllithium in ether was added to a solution of 27 g (0.2 moles) of thianaphthene in 100 ml of dry ether. The reaction was kept at about −10° during the addition. The mixture was then stirred at room temperature for one hour and refluxed for one hour.

2-Thianaphthenylmagnesiumbromide (II)

The lithium compound was converted to the Grignard compound with magnesium bromide etherate (14). An ether solution of anhydrous magnesium bromide, prepared by adding 17 ml (0.3 mole) of bromine dropwise to 8.5 g (0.35 mole) of magnesium suspended in 250 ml of ether, was added to the lithium compound (I). The mixture was stirred at room temperature for half an hour.

α-Hydroxy-α-(2-thianaphthenyl)-propionic acid (III)

The solution containing the Grignard compound (II) was added with stirring and cooling to 29 g (0.25 mole) of ethyl puruvate (1) in 200 ml of ether. The temperature was kept at about −10°. The Grignard reagent reacted vigorously with the ester and a white precipitate separated. The mixture was kept cold for 3 hours after the addition and then stirred for a further 3 hours at room temperature. The reaction mixture was hydrolyzed with ice and dilute acetic acid. The organic layer was separated and the water extracted with ether. A crystalline product, which was suspended in the water phase, was filtered off. The combined ether extracts were transferred to a flask, the ether evaporated and the residue saponified with 10 % potassium hydroxide. The alkaline water solution was extracted with ether and then acidified. The acid separated as an oil which immediately crystallized. It was recrystallized once from benzene and once from carbon tetrachloride. Yield 14 g (40 % counted on reacted thianaphthene), m.p. 142–144°.

Anal. $C_{11}H_{10}O_3S$ (222,27):
Calc. equiv. wt. 222.3; C 59.44; H 4.53; S 14.43.
Found equiv. wt. 223.2; C 59.34; H 4.61; S 14.47.

Six g of thianaphthene was recovered from the ether extract of the alkaline water solution.

The crystalline product first filtered off from the water phase was recrystallized from dimethyl sulphoxide. Yield 2.0 g of 2,2′-dithianaphthenyl melting at 261–262°. Fries and Hemmecke reported m.p. 262° for 2,2′-dithianaphthenyl (15). Shirley and Cameron obtained this compound as a by-product when they reacted 2-thianaphthenyllithium with bromine (13).

α-(2-Thianaphthenyl)-propionic acid (IV)

The acid (III) was reduced in the same way as the α-hydroxy-α-(3-thianaphthenyl)-propionic acid (1). The only difference was that some ml of 57 % hydriodic acid was present. The reduced acid was recrystallized once from formic acid and once from carbon tetrachloride. Yield 65 %, m.p. 117.5–119°.

Anal. $C_{11}H_{10}O_2S$ (206.27):
Calc. equiv. wt. 206.3; C 64.04; H 4.88; S 15.54.
Found equiv. wt. 207.1; C 64.08; H 4.96; S 15.56.

Resolution of the racemic acid

Preliminary experiments were carried out with different bases and solvents. The crystals formed were filtered off, the acid liberated and the optical activity measured in absolute ethanol. The results are given in Table 1.

Table 1. Preliminary tests on the resolution of α-(2-thianaphthenyl)-propionic acid.

Base	$[\alpha]_D$ of acid in abs. ethanol			
	From methanol	From ethanol	From acetone	From ethyl acetate
Cinchonine	oil	oil	oil	+ 2°
Cinchonidine	− 2°	+13°	+ 4°	+ 4°
Quinine	± 0	± 0	± 0	± 0
Quinidine	oil	oil	oil	oil
Brucine	− 2°	± 0	− 2°	± 0
Strychnine	oil	oil	oil	oil
Morphine	+23°	± 0	+20°	+26°
Ephedrine	oil	oil	oil	oil
(+)-α-Phenylethylamine	− 2°	− 3°	± 0	± 0
(+)-α-(2-Naphthyl)-ethylamine	−21°	− 8°	− 5°	−26°

Table 2. Recrystallization of the morphine salt of α-(2-thianaphthenyl)-propionic acid.

Cryst. no.	Solvent (ml)		Weight of salt (g)	$[\alpha]_D^{25}$ of acid
	Ethanol	Water		
1	600	150	28.1	25°
2	400	100	20.0	30°
3	300	75	16.6	31°
4	250	60	14.5	31°

A mixture of 20.6 g (0.1 mole) of racemic acid and 30.3 (0.1 mole) of morphine was dissolved in a hot mixture of 600 ml of ethanol and 150 ml of water. The salt was filtered off after 24 hours and recrystallized from dilute ethanol. The progress of the resolution was tested on samples from each crystallization, from which the acid was liberated and the optical activity examined in absolute ethanol (Table 2).

The mother liquors from the two first crystallizations were evaporated to dryness at room temperature and 13.5 g acid was isolated with $[\alpha]_D^{25} = -18°$ (absolute ethanol). This acid was added to an ether solution containing about 11.2 g of (+)-α-(2-naphthyl)-ethylamine obtained from 15.6 g of the sulfate (16, 17). The ether was evaporated and the salt recrystallized from dilute ethanol. The progress of the resolution was followed as described above and the results are given in Table 3.

Table 3. Recrystallization of the (+)-α-(2-naphthyl)-ethylamine salt of α-(2-thianaphthenyl)-propionic acid.

Cryst. no.	Solvent (ml)		Weight of salt (g)	$[\alpha]_D^{25}$ of acid
	Ethanol	Water		
1	600	120	20.0	−27°
2	500	100	16.2	−31°
3	400	80	14.0	−31°

(+)-α-(2-*Thianaphthenyl*)-*propionic acid*

The acid was liberated from the morphine salt with dilute sulphuric acid and extracted with ether. The ether was evaporated and the crude acid (6.1 g) was recrystallized from a carbon tetrachloride–ligroin mixture. Yield 5.4 g of acid forming small needles with m.p. 134–136°.

Anal. $C_{11}H_{10}O_2S$: Equiv. wt. calc. 206.3; found 207.0.

Optical activity in different solvents

Weighed amounts of the acid were dissolved in different solvents, made up to a volume of 10.00 ml and the rotatory power measured. In the case of water the acid was neutralized with dilute sodium hydroxide to pH 7. The results are given in Table 4.

Table 4. Optical activity of (+)-α-(2-thianaphthenyl)-propionic acid.

Solvent	mg of acid	$2\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
Acetone	100.4	+1.04°	+51.8°	+106.9°
2.2.4-Trimethylpentane	15.5	+0.14°	+45°	+ 93°
Acetic acid	101.0	+0.71°	+35.2°	+ 72.6°
Ethanol	105.8	+0.66°	+31.2°	+ 64.4°
Chloroform	100.8	+0.44°	+21.8°	+ 45.0°
Benzene	100.8	+0.28°	+13.9°	+ 28.7°
Water (ion)	100.6	−0.53°	−26.3°	− 54.3°

(−)-α-(2-Thianaphthenyl)-propionic acid

The naphthylethylamine salt (13.8 g) was decomposed and the acid isolated in the same way as for the (+)-antipode. 7.5 g of the acid was obtained. It was recrystallized from carbon tetrachloride-ligroin. Yield 7.0 g; m.p. 134–136°.

Anal. $C_{11}H_{10}O_2S$: Equiv. wt. calc. 206.3; found 206.7.
0.1002 g of acid dissolved in absolute ethanol to 10.00 ml: 2 $\alpha_D^{25} = -0.63°$, $[\alpha]_D^{25} = -31.4°$, $[M]_D^{25} = -64.8°$.

Desulphurization of (+)-α-(-2-thianaphthenyl)-propionic acid

A mixture of 2.1 g (0.01 mole) of (+)-α-(2-thianaphthenyl)-propionic acid, 2.5 g of sodium carbonate, 300 ml of distilled water and 40 g of Raney nickel catalyst (18) was heated at 80° with stirring for two hours. The nickel was filtered off, the catalyst washed with dilute carbonate solution, and the filtrate acidified with sulphuric acid and extracted with ether. After evaporation of the ether there remained a slightly yellow oil. The specific rotation in absolute ethanol was +23.5°. The oil was distilled in vacuum. Yield 1.6 g with b.p. 150–160° at 2 mm.

Anal. $C_{11}H_{14}O_2$ (178.23):
Calc. equiv. wt. 178.2; C 74.13; H 7.92; S 0.
Found equiv. wt. 180.0; C 72.52; H 8.07; S 0.09.
0.1736 g of acid dissolved in aboslute alcohol to 10.00 ml: 2 $\alpha_D^{25} = +0.82°$, $[\alpha]_D^{25} = +23.6°$, $[M]_D^{25} = +42.1°$. In homogeneous state $[\alpha]_D^{25} = +26°$, $[M]_D^{25} = +46°$.

Ozonolyses of (+)-α-methyl-γ-phenylbutyric acid

A 1.4 g sample of the (+)-α-methyl-γ-phenylbutyric acid obtained was dissolved in a mixture of 25 ml of acetic acid and 25 ml of ethyl acetate. The solution was treated with a stream of oxygen-ozone at about −15° for two hours. The solvent was then evaporated *in vacuo* and the ozonide dissolved in 10 ml of acetic acid. The ozonide was decomposed by adding 10 ml of perhydrol and heating at 40° for 36 hours and finally boiling for some minutes. The solvent was evaporated *in vacuo* and the product dissolved in 30 ml of 1-molar sulphuric acid. Potassium permanganate was added until the permangante colour persisted. The suspension was filtered and the aqueous solution thoroughly extracted with ether. The ether was evaporated and the acid recrystallized from a benzene–ligroin mixture. Yield 0.5 g; m.p. 71–81°. Mixed melting point with authentic (+)-α-methylglutaric acid was 73–82°. Infrared spectra were identical.

Anal. $C_6H_{10}O_4$: Equiv. wt. calc. 73.1; found 73.3.
0.1500 g of acid dissolved in chloroform to 10.00 ml: 2 $\alpha_D^{25} = +0.70°$, $[\alpha]_D^{25} = +23.3°$, $[M]_D^{25} = +34.1°$.

SUMMARY

α-Hydroxy-α-(2-thianaphthenyl)-propionic acid has been prepared and reduced to α-(2--thianaphthenyl)-propionic acid. The latter acid has been resolved into the optical antipodes using morphine and (+)-α-(2-naphthyl)-ehylamine. The rotatory power found was $[\alpha]_D^{25} = +31.2°$ and $[\alpha]_D^{25} = -31.4°$ respectilvely (in absolute ethanol).

The configuration has been established by desulphurization of (+)-α-(2-thianaphthenyl)-propionic acid with Raney nickel to (+)-α-methyl-γ-phenylbutyric acid followed by ozonolysis of the latter acid to (+)-α-methylglutaric acid. The reaction sequence indicates that α-(2-thianaphthenyl)-propionic acid and α-methyl-γ-phenylbutyric acid with the same direction of rotation also have the same configuration and that the (+)-forms are related to D-glyceraldehyde.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Professor Arne Fredga for valuable discussions and for the authentic samples of optically active α-methylglutaric acid. I am also indebted to Mr. Stig Bergvall for taking the IR spectra referred to.

A grant from the *Swedish Natural Science Research Council* is gratefully acknowledged.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, November 1957.

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Tryckt den 3 juni 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 50

Communicated 12 March 1958 by Arne Fredga

Stereochemical studies. V

The enantiomers of α-(4-methyl-1-naphthyl)-propionic acid

By Berndt Sjöberg

When the structure of two different types of compounds, both having auxin activity, is changed in the same way, the change in auxin activity of the two compounds may be quite different. Phenoxyacetic acid is a weak antiauxin. When a hydrogen at the α-carbon atom is replaced by a methyl group the resulting propionic acid turns out to be a weak auxin. The same type of change will occur when a methyl group is introduced in the para-position (1). Phenylacetic acid, which is a weak auxin, will be a still weaker auxin when a hydrogen at the α-carbon or at the para-carbon is replaced by a methyl group (2). The very active 1-naphthylacetic acid will have its activity reduced when it is substituted in an analogous way, but α-(1-naphthyl)--propionic acid as well as 4-methyl-1-naphthylacetic acid have pronounced activity (2, 3). It therefore seemed to be of interest to see, if methyl substitution in the 4-position would simply reduce the activity of α-(1-naphthyl)-propionic acid or completely change the type of plant growth regulating activity.

The racemic α-(4-methyl-1-naphthyl)-propionic acid (VI) was prepared in an analogous way as α-(1-naphthyl)- and α-(2-naphthyl)-propionic acid (3, 4) according to the following general procedure:

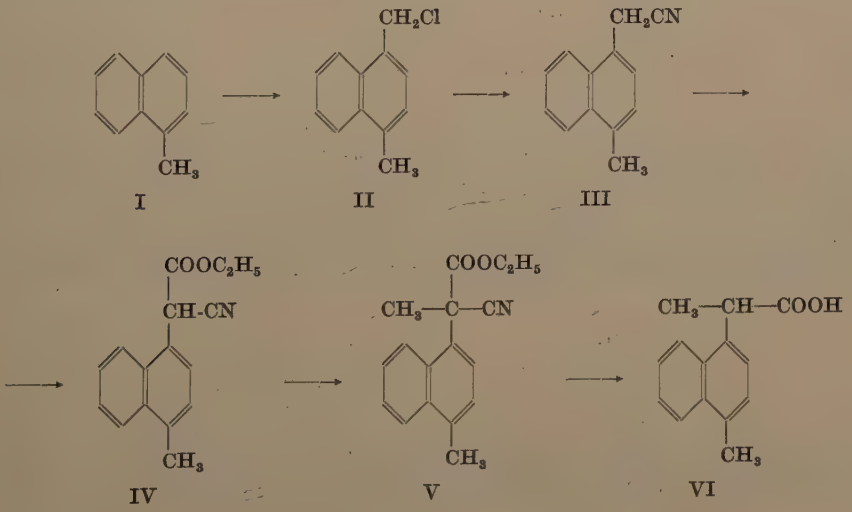


Table 1. $[M]_D^{25}$ for (−)-α-(4-methyl-1-naphthyl)-propionic acid (MNP) and (+)-α--(1-naphthyl)-propionic acid (NP).

Solvent	$[M]_D^{25}$ MNP	NP
2,2,4-Trimethylpentane . .	−336.7°	342.4°
Benzene	−331.7°	361.5°
Acetic acid	−297.0°	304.8°
Acetone	−277.5°	292.1°
Chloroform	−247.7°	268.2°
Ethanol	−233.5°	240.9°
Water (ion)	− 47.4°	69.9°

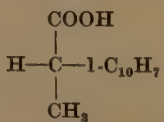
Chloromethylation of 1-methylnaphthalene (I) to 1-chloromethyl-4-methylnaphthalene (II) has been described by Darzens and Lévy (5), but some modifications improved the result. The chloromethyl compound was converted in good yield to 4-methyl-1-naphthylacetonitrile (III). This substance was obtained as crystal needles (m.p. 78–79°) and not as an oil (6).

The resolution of the racemic acid (VI) was performed with quinine and cinchonidine. The dextrorotatory acid was obtained by recrystallization of the quinine salt from ethyl acetate. The cinchonidine salt gave the levorotatory form from dilute acetone.

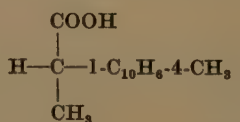
The optical activity was determined in different solvents and compared with that of α-(1-naphthyl)-propionic acid. The results are given in Table 1. A certain parallelism is found, indicating that the acids with the same direction of rotation also have the same configuration.

A definite proof for the steric relations between the two acids was obtained from the melting point diagrams. Considering the structural similarity of α-(1-naphthyl)- and α-(4-methyl-1-naphthyl)-propionic acid and the great racemate-formation tendency of the two acids, a quasi-racemate could be expected between the acids. A very pronounced quasi-racemic compound was also formed from the acids with opposite direction of rotation. The antipodes with the same direction of rotation gave a simple eutectic diagram. The diagrams will soon be published in a following paper in this journal.

It can thus be concluded that α-(4-methyl-1-naphthyl)-propionic acid and α-(1--naphthyl)-propionic acid with the same direction of rotation also have the same configuration. The configuration of α-(1-naphthyl)-propionic acid has been determined by Fredga and the (+)-form has been shown to have the configuration VII (3). The dextrorotatory α-(4-methyl-1-naphthyl)-propionic acid consequently has the configuration represented by the stereoformula VIa and is thus related to D-glyceraldehyde.



VII



VI a

The plant physiological properties of 4-methyl-1-naphthylacetic acid and of α-(4-methyl-1-naphthyl)-propionic acid have been studied by Professor B. Åberg who has kindly put some preliminary results at the authors disposal. The 4-methyl-1-naphthylacetic acid is a typical auxin but has lower activity than 1-naphthylacetic acid. On the other hand, replacement of the hydrogen in the 4-position by methyl has essentially changed the type of plant growth regulating activity of α-(1-naphthyl)--propionic acid. Both the antipodes of α-(1-naphthyl)-propionic acid are auxins with a distinct difference in activity between the antipodes, while α-(4-methyl-1-naphthyl)--propionic acid is a weak antiauxin. In the *Avena cylinder test* and in the *wheat root test* there is no difference in activity between the antipodes. In the *flax root test* the (−)-antipode is slightly more active than the (+)-antipode (7).

Experimental

1-Methylnaphthalene (*I*)

The substance was commercial, Eastman Kodak Company, and of purity classification "practical grade".

1-Chloromethyl-4-methylnaphthalene (*II*)

The preparation was performed in a similar way to the chloromethylation of naphthalene described in Organic Syntheses (8). In a 1-l flask, fitted with a reflux condenser and a stirrer, were placed 142 g (1 mole) of 1-methylnaphthalene, 55 g of paraformaldehyde, 130 ml of glacial acetic acid, 85 ml of 85 % phosphoric acid and 180 ml of concentrated hydrochloric acid. The mixture was heated at 55° for six hours. After the mixture had been cooled, 500 ml of ice-water and 300 ml of ether were added. The organic layer was separated and washed with water, then with cold sodium carbonate solution, and finally with cold water. The solution was dried with potassium carbonate, 100 ml of benzene was added and the solvent removed. The product was fractionated in a short column. Yield 140 g (74 %) boiling at 126–127°/2 mm. The product immediately solidified (m.p. 59–62°). Darzens and Lévy reported the boiling point to 124–125°/2 mm and the melting point to 62° (5).

1-Cyanomethyl-4-methylnaphthalene (*III*)

In a 1-l flask, equipped with a reflux condenser and a stirrer, was placed 65 g (1 mole) of potassium cyanide and 5 g of potassium iodide in 150 ml of water. A solution of 96 g (0.5 mole) of the chloromethyl compound (II) in 400 ml of acetone was added. The mixture was refluxed for six hours. Water was added and the acetone distilled off. The product was extracted with ether, and the ether extract dried and fractionated. The cyanomethyl compound, which boiled at 163–165°/2 mm, immediately solidified. It was recrystallized from ligroin (b.p. 85–110°). Yield 82 g (85 %) with m.p. 78–79°. Buu-Hoi and Cagniant reported b.p. 154–156°/0.5 mm (6).

Anal. $C_{13}H_{11}N$ (181.24):
Calc. C 86.15; H 6.12.
Found C 86.10; H 6.18.

4-Methyl-1-naphthylacetic acid

A small part of the nitrile (III) was saponified with alcoholic potassium hydroxide. The acid obtained, which was recrystallized from formic acid, had a melting point of

177–178°. Buu-Hoi and Cagniant reported the melting point 148° (6). The same melting point has also been reported by Melnikow (2). In 1951 Lettré and Stratmann prepared the acid in the above way and their product melted at 178° (9). Attempts to get the modification melting at 148° were unsucessful. However, when the acid was melted and rapidly cooled a modification crystallized which was transformed at 65–68° and finally melted at 177–178°.

Anal. $C_{13}H_{12}O_2$: Equiv. wt. calc. 200.2; found 199.6.

Ethyl-(4-methyl-1-naphthyl)-cyanoacetate (IV)

In a 1-l flask, fitted with a reflux condenser, was placed 90.5 g (0.5 mole) of the nitrile (III), 250 ml of diethyl carbonate and 11.5 g (0.5 mole) of sodium. The flask was heated in an oil bath at 100–110° and the reaction was completed in half an hour. The reflux condenser was replaced by a short column and the alcohol produced by the reaction was distilled off. After cooling the reaction mixture was acidified with dilute acetic acid. The organic layer was separated and the water extracted with ether. The combined organic layer and ether extract was washed with sodium carbonate solution and water, dried and fractionated. The cyanoacetate boiling at 191–193°/2 mm soon solidified. It was recrystallized from ethanol. Yield 100 g (79 %), m.p. 104–106°.

Anal. $C_{16}H_{15}O_2N$ (253.30):
Calc. C 75.87; H 5.97.
Found C 75.90; H 6.01.

Ethyl-(4-methyl-1-naphthyl)-cyanopropionate (V)

In a 1-l flask, fitted with a reflux condenser, was placed 300 ml of absolute ethanol and 8.3 g (0.36 mole) of sodium. To the alcoholate were added 89 g (0.35 mole) of the cyanoacetate (IV) and 71 g (0.5 mole) of methyl iodide. The mixture was refluxed for three hours. The alcohol was distilled off *in vacuo*, and the residue was shaken with potassium hydroxide solution and ether. Part of the cyanopropionate crystallized and was filtered off. The ether was evaporated and the residue recrystallized from alcohol. 13 g of cyanoacetate was recovered from the alkaline water phase by acidification. Yield 51 g (63 % calculated on reacted cyanoacetate), m.p. 115–117°.

Anal. $C_{17}H_{17}O_2N$ (276.33):
Calc. C 76.37; H 6.42.
Found C 76.23; H 6.49.

α-(4-Methyl-1-naphthyl)-propionic acid (VI)

In 300 ml of alcohol were dissolved 60 g of the ester (V) and 30 g of potassium hydroxide. The mixture was refluxed for one hour, water was added and the mixture refluxed for another 24 hours. The alcohol was distilled off and the water extracted with ether. The water was acidified and 44 g of an acid melting at 118–122° was obtained. The acid was recrystallized once from formic acid and once from ligroin (b.p. 85–110°). Yield 34 g (71 %), m.p. 125–127°.

Anal. $C_{14}H_{14}O_2$ (214.27):
Calc. equiv. wt. 214.3; C 78.48; H 6.59.
Found equiv. wt. 214.4; C 78.29; H 6.58.

Table 2. Preliminary tests on the resolution of α-(4-methyl-1-naphthyl)-propionic acid.

Base	$[\alpha]_D^{25}$ of acid in abs. ethanol			
	From methanol	From ethanol	From acetone	From ethyl acetate
Cinchonine	± 0°	± 0°	+17°	oil
Chinconidine	−18°	−28°	−47°	−18°
Quinine	oil	oil	oil	+37°
Quinidine	oil	oil	oil	oil
Brucine	+12°	− 3°	− 4°	− 9°
Strychnine	oil	oil	oil	oil
Morphine	oil	+ 2	oil	oil
(+)-α-Phenylethylamine	oil	oil	oil	oil

Table 3. Recrystallization of the quinine salt of α-(4-methyl-1-naphthyl)-propionic acid.

Cryst. no.	Ethyl acetate (ml)	Weight of salt (g)	$[\alpha]_D^{25}$ of acid
1	100	15.6	69°
2	80	12.5	90°
3	60	10.6	102°
4	50	9.3	106.5°
5	50	8.0	108°
6	50	7.0	108°

Resolution of the racemic acid

Preliminary experiments were carried out with 0.001 mole of the racemic acid and 0.001 mole of the bases in different solvents. As can be seen in Table 2 the best results were obtained from quinine and cinchonidine.

In a hot solution of 100 ml of ethyl acetate were dissolved 12.8 g (0.06 mole) of racemic acid and 19.5 g (0.06 mole) of quinine. The salt was allowed to crystallize at room temperature and then in a refrigerator. The salt was filtered off after 24 hours and recrystallized from ethyl acetate. The progress of the resolution was tested on small samples from each crystallization, from which the acid was isolated and the optical activity measured in absolute ethanol (Table 3).

The mother liquors from the three first crystallizations were evaporated to dryness and 8.4 g of acid was isolated with $[\alpha]_D^{25} = -29°$ (absolute ethanol). This acid was dissolved with 11.4 g of cinchonidine in a boiling mixture of 180 ml of acetone and 18 ml of water. The salt was recrystallized from dilute acetone until the optical activity of the acid remained constant (Table 4).

(+)-α-(4-Methyl-1-naphthyl)-propionic acid

The acid was liberated from 6.5 g of the salt with dilute sulphuric acid and extracted with ether. The yield of the crude acid was 2.5 g and after two recrystallizations from formic acid there remained 2.0 g with m.p. 77–78°.

Table 4. Recrystallization of the cinchonidine salt of α-(4-methyl-1-naphthyl)-propionic acid.

Cryst. no.	Solvent (ml) Acetone	Water	Weight of salt (g)	$[\alpha]_D^{25}$ of acid
1	180	18	13.5	− 83°
2	100	10	10.3	−102°
3	100	10	8.5	−106°
4	100	10	6.8	−108°
5	100	10	5.9	−108°

Table 5. Optical activity of (−)-α-(4-methyl-1-naphthyl)-propionic acid.

Solvent	mg of acid	$2\,\alpha_D^{25}$	$[\alpha]_D^{25}$	$[M]_D^{25}$
2,2,4-Trimethylpentane . . .	64.6	−2.04°	−157.1°	−336.7°
Benzene	67.5	−2.09°	−154.8°	−331.7°
Acetic acid	69.6	−1.93°	−138.6°	−297.0°
Acetone	66.4	−1.72°	−129.5°	−277.5°
Chloroform	74.8	−1.73°	−115.6°	−247.7°
Ethanol	76.1	−1.66°	−109.0°	−233.6°
Water (ion)	72.4	−0.32°	− 22.1°	− 47.4°

Anal. $C_{14}H_{14}O_2$: Equiv. wt. calc. 214.3; found 214.5.

0.0643 g acid dissolved in absolute ethanol to 10.00 ml: $2\,\alpha_D^{25} = +1.39°$, $[\alpha]_D^{25} = +108.5°$, $[M]_D^{25} = +232.4°$.

(−)-α-(4-Methyl-1-naphthyl)-propionic acid

From 5.6 g of the cinchonidine salt 2.3 g of acid was isolated. It was recrystallized twice from formic acid. Yield 1.8 g, m.p. 77–78°.

Anal. $C_{14}H_{14}O_2$: Equiv. wt. calc. 214.3; found 214.4.

Optical activity in different solvents

Weighed amounts of the acid were dissolved in different solvents, made up to a volume of 10.00 ml and the rotatory power determined. In the case of water the acid was neutralized with dilute sodium hydroxide to pH 7 (Table 5.)

Melting point diagrams

Weighed amounts of the components were dissolved in pure acetone. The solvent was evaporated, the residue carefully powdered and dried. The thaw-melting point determinations were carried out using a hot stage microscope according to Kofler (10). The diagrams will be published in a following paper in this journal.

SUMMARY

α-(4-Methyl-1-naphthyl)-propionic acid has been prepared and resolved into the optical antipodes. The steric relationship of the acid to α-(1-naphthyl)-propionic acid has been estiablished by the quasi-racemate method. Preliminary experiments on the plant growth regulating activity of the enantiomers are reported.

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Professor Arne Fredga for his interest in this work and for the antipodes of α-(1-naphthyl)-propionic acid kindly placed at the author's disposal. The author is also indebted to Professor Börje Åberg, Royal Agricultural College, Uppsala, for kindly supplying the preliminary results on the plant growth regulating activity.

A grant from the *Swedish Natural Science Research Council* is gratefully acknowledged.

Department of Organic Chemistry, Chemical Institute, University of Uppsala, November 1957.

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Tryckt den 3 juni 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB

ARKIV FÖR KEMI Band 12 nr 51

Communicated 12 February 1958 by Karl Myrbäck and Gunnar Blix

Studies on the transformation and degradation of sialic acids in aqueous solution

By Ingemar Berggård and Lars Odin

With 10 figures in the text

Sialic acids are characterized by a marked instability in aqueous solution (1). On heating in acid solution they are rapidly decomposed under humin formation. At an alkaline pH they are degraded to compounds such as 2-carboxypyrrole (2, 3, 4), N-acetyl-D-glucosamine (5, 6), and pyruvic acid (5). The present investigation was undertaken to obtain more detailed information of the changes that the sialic acids undergo in aqueous solution on variation of pH and temperature. The changes were followed mainly by studying the ultraviolet absorption and the behaviour on chromatography.

The identification of 2-carboxypyrrole, as a degradation product of sialic acids, has up to now mainly been based on paper chromatographic and spectroscopic evidence (3, 4). As part of the present study we also thought it of importance to isolate the substance in crystalline form and finally settle the identity.

Experimental

Materials

Sialic acids.—Recrystallized preparations of N-glycolyl, N-acetyl, and N,O-diacetyl neuraminic acids (P-, O-, and B-sialic acids) as well as the methyl esters of the two former substances were used in this study. The compounds were isolated by the procedure described by Blix *et al.* (7), and showed the elementary composition and X-ray diffraction patterns reported by these authors. N-acetyl neuraminic acid was also isolated by the method of Zilliken *et al.* (8). This was done to exclude the possibility of methyl ester formation. (In the Zilliken procedure acetic acid is used instead of methanol in the crystallization step.).

N-acetyl-D-glucosamine.—A specimen prepared by Prof. Blix was kindly put to our disposal.

2-carboxypyrrole was synthesized by Oddo's method as modified by McCay and Schmidt (9). The substance gave one single spot on paper chromatography in different solvents. M.p. 190–192°.

Ion exchange resin.—Dowex 1, ×2, 200–400 mesh, was employed. To obtain uniform particle size, the smallest and the largest particles were removed by sedimentation. The resin was washed and converted to formate form (10).

Methods

Preparation of test solutions

0.01 *M* solutions of the test substances were used. The solvents were:

(I) a buffer series, covering the pH-range from 2 to 10 with intervals of about one unit, including Sörensen's citrate and phosphate mixtures, and a 0.1 *M* sodium carbonate–bicarbonate buffer. In some experiments the citrate–borate–phosphate–HCl system of Teorell and Stenhagen (11) was also used in the pH-range from 2 to 11. 0.1 *N* sodium hydroxide was added to the buffered solutions in an amount equivalent to the content of sialic acid.

(II) acids such as 1 *N* acetic acid, 0.1 *N* hydrochloric and sulphuric acid, and distilled water, and a water suspension of Dowex 50, H (both being acid after addition of sialic acid).

(III) 0.02, 0.04, and 0.2 *N* barium hydroxide and 0.1 *N* sodium hydroxide as additional alkaline solvents.

The pH-values of the buffered solutions were checked in a Beckman pH-meter before further treatment. Changes in the pH, occurring on heating, were not determined.

The test solutions were kept in glass-stoppered tubes, and heated in water of constant temperature.

When sulphuric acid and barium hydroxide were used the solutions were neutralized with baryta and carbon dioxide, respectively, prior to chromatography. Otherwise no neutralization or desalting was performed.

Spectrophotometry

A Beckman DU spectrophotometer with a hydrogen discharge lamp, and 10 mm silica cells were used for the ultraviolet examination. The wavelength scale of the apparatus was calibrated by means of a mercury lamp. The light absorption of the test solutions in the near ultraviolet range, 210–300 mμ, was measured after standing at room temperature, and after heating at 40, 70, and 100°C for different periods of time. Solvent without test substance was used as blank.

Paper chromatography

Samples, corresponding to an initial content of the test substance of 0.2–0.6 mg, were applied to the paper strips. Chromatography was carried out using the descending technique and *n*-butanol–acetic acid–water (BA) (4:1:5 v/v), and *n*-butanol–pyridine–water (BP) (6:4:3 v/v) as solvents. The papers were sprayed with Ehrlich's reagent—*p*-dimethylaminobenzaldehyde-hydrochloric acid (*p*-DAB) (12)—and heated at 105°C for a few minutes. This reagent gives purple or violet colours with sialic acids, N-acetylhexosamines, and some of their degradation products. Orcinol-trichloroacetic acid (13) and aniline hydrogen phthalate (14) were also used as spraying reagents. The R_f-determinations were made exclusively on Whatman paper No. 1.

In some experiments the separated fractions were eluted from the paper for further investigation. The paper sheets (Munktell OB) were in these cases thoroughly washed with acetic acid, water, and ethanol, and dried before use. The fractions were located by staining narrow strips, which were cut off both sides or out of the center part of the paper sheet. The areas of the sheet, corresponding to the colour spots of the test strips, were cut out, and the material eluted with the appropriate solvent. The papers were then sprayed with colour reagent to check complete elution. Sections of similar size of paper sheets, run with the same solvent, but without any test material applied, were used as "paper blanks" in quantitative determinations.

Isolation of 2-carboxypyrrole from N-glycolyl neuraminic acid methyl ester

500 mg of the ester was dissolved in 25 ml of baryta (saturated at room temperature). The solution was heated under reflux for one hour in a boiling water bath. It was then cooled and neutralized with 1 *N* sulphuric acid to approximately pH 7. After centrifuging the supernatant was decanted and freeze-dried. The brownish residue was dissolved in a few ml of distilled water, filtered, and applied to a Dowex 1 column in formate form, 1.0 × 20 cm. After washing the column with 200 ml of water, the adsorbed material was eluted with formic acid, using the gradient elution technique, the final concentration of the eluent being 1 *N*. 10 ml fractions were collected. A small sample of every second fraction was examined with the "direct" Ehrlich reaction. Two distinct peaks were obtained of material, that gave a very intense purple colour with *p*-DAB at room temperature, and a still deeper one on heating. (All the remaining tubes contained small amounts of Ehrlich-positive material.) The effluents of each of the two fractions were pooled and lyophilized. On paper chromatography using BA, both fractions appeared as distinct spots, giving purple colours with the Ehrlich and orcinol reagents. The first column-fraction had an R_f-value of approximately 0.60. The second column-fraction showed an identical R_f-value, 0.84, and produced the same colour shade as an authentic specimen of 2-carboxypyrrole. The lyophilized second fraction was dissolved in a small volume of water at room temperature. After a few days crystals began to appear. The crystalline material was dissolved in aqueous ethanol and recrystallized on standing. It was then rapidly washed with water and again crystallized from aqueous ethanol, and finally dried in a desiccator. The final yield was about 2 mg of crystalline material. (The mother liquor still contained considerable amounts of Ehrlich-positive chromogens. No attempts were made, however, to obtain better yields, as our purpose only was to get enough material for identification.) The X-ray diffraction of the crystalline material was found to be identical with that of 2-carboxypyrrole. (The X-ray powder diagrams were taken with a Guinier camera, using nickel-filtered copper-Kα-radiation.)

Results

Ultraviolet absorption of unheated solutions

Sialic acids in aqueous solution of pH between 2.0 and 10.0 show a simple end-absorption down to 210 mμ. (In many experiments there was noted a very low absorption in the region of 260–290 mμ without a distinct maximum, possibly due to some impurity.) No changes are found after standing for 24 hours.

The methyl esters of the N-glycolyl and N-acetyl acids show an ultraviolet absorption, at pH 6.5–9, with a maximum at about 265 mμ, initially rapidly increasing with time (Fig. 1). The maximal optical density of the transient absorption was obtained at approximately pH 8. In phosphate buffer of this pH maximum absorption was reached after 20–25 minutes, at a less alkaline pH somewhat later. Thereafter the absorption decreased, at pH 8 approaching the initial values within 2 hours. (In the borate–citrate–phosphate system the absorption was weaker, appeared later, and decreased more slowly than in the phosphate and carbonate buffers.) On acidification there occurred an instant drop of absorption which was almost completely and instantaneously reversed by the addition of alkali. At a pH above 9 an absorption with maximum at about 265 mμ was observed, which showed no initial rise and

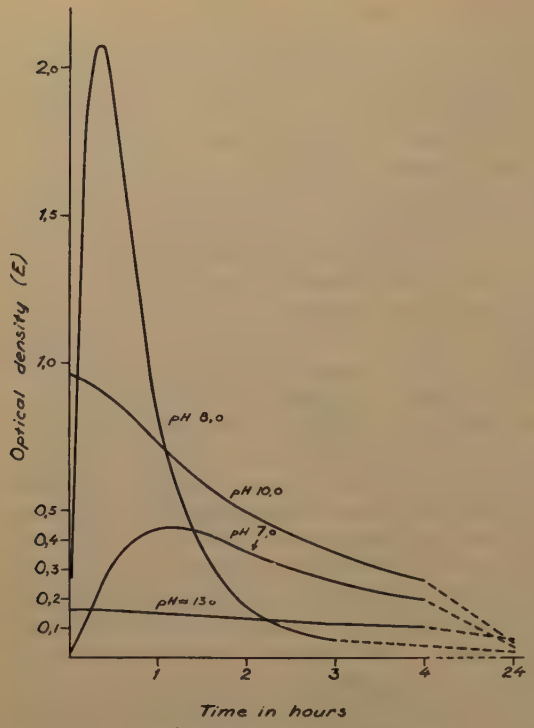

Fig. 1. Ultraviolet absorption at 265 mμ of 0.001 *M* N-acetyl neuraminic acid methyl ester in aqueous solutions of different pH, measured at different time intervals after the substance had been dissolved.

decreased more slowly than the absorption at pH 8. The highest initial values were found at the lower pH's.

Some of the sialic acid preparations exhibited the same absorption characteristics but to a lesser extent, 1–2 % of that of the esters. Methoxyl determinations indicated the presence in these substances of small quantities of methyl ester. A completely methoxyl-free preparation did not develop a measurable transient absorption at pH 8.

Ultraviolet absorption after heating

Heating the sialic acids at 100°C in 0.1 *N* hydrochloric acid for 15–30 minutes gave rise to a strong absorption over the whole wave length range measured, with a small peak at 270 mμ. Marked humin formation occurred. A similar effect was noted when sialic acids dissolved in distilled water or in buffers of *pH 2–4* were heated for one hour at 100°C (Fig. 2). The absorption at 270 mμ increased with the acidity of the solution as did the humin formation.

In the *pH-range 4–5* the original absorption curves were very little changed after heating at 100°C for one hour (Fig. 2). At pH 4.6 no distinct absorption band was obtained even after heating for 13 hours.

At $pH > 5$ heating at 100°C for one hour resulted in the appearance of an absorption peak at about 260 mμ (257–261 mμ), the intensity of which increased with increasing pH (Fig. 2). (In 0.1 *N* sodium hydroxide the intensity was about four times greater than that measured at pH 9.2.)

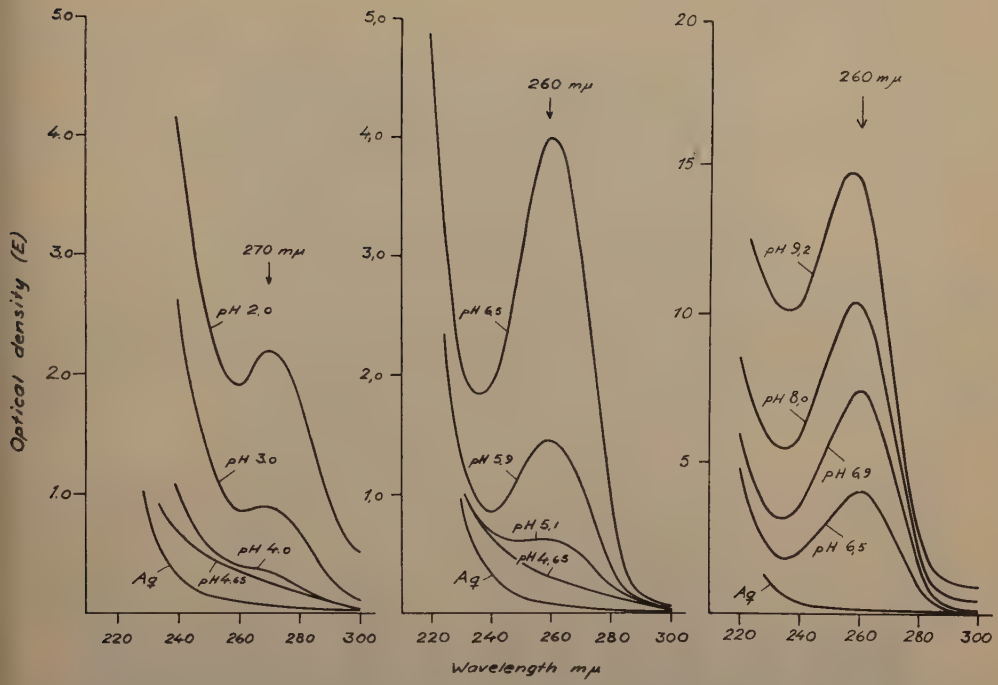

Fig. 2. Ultraviolet absorption spectra of 0.01 *M* N-glycolyl neuraminic acid in aqueous solutions of various pH after heating at 100°C for 1 hour. The curve designated *Aq* represents an unheated solution of the same substance in distilled water.

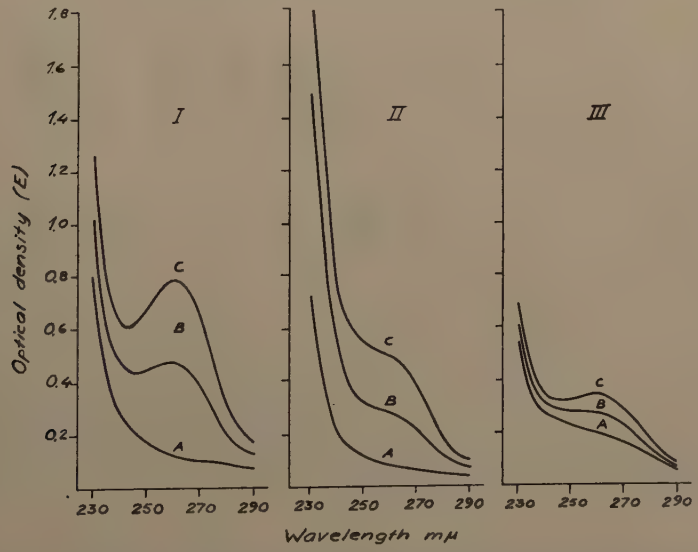

Fig. 3. Ultraviolet absorption curves of 0.01 *M* solutions of different sialic acids in phosphate buffer, pH 8, heated at 40°C (A) for 1 hour, (B) 24 hours, and (C) 48 hours respectively. (I) N-glycolyl, (II) N,O-diacetyl, and (III) N-acetyl neuraminic acid.

Heating at lower temperature, 40 and 70°C respectively, gave principally the same results, but longer heating times were required to get well-defined adsorption peaks (Fig. 3).

The absorption at 260 mμ on heating at pH > 5 was more intense and developed more rapidly with solutions of N-glycolyl neuraminic acid than with those of the N-acetyl variety. The bovine diacetyl acid gave intermediate values.

Molecular extinction coefficients of 2-carboxypyrrole

2-carboxypyrrole in distilled water showed an ultraviolet absorption with maximum at 262 mμ and $\varepsilon = 12{,}900$. In phosphate buffer pH 8: λ max 257 mμ, $\varepsilon = 12{,}300$; in citrate buffers pH 4.7: λ max 260 mμ, $\varepsilon = 12{,}300$; and pH 3.0: λ max 267 mμ, $\varepsilon = 14{,}700$. The absorption was essentially unchanged after heating at 100°C for one hour in phosphate buffer, pH 8, and in 0.1 *N* sodium hydroxide, but decreased rapidly on heating in acid solution—citrate buffer, pH 3, and 0.1 *N* hydrochloric acid.

Paper chromatography

Chromatograms of sialic acids, heated in aqueous solvents of varying pH, are shown in Figs. 4–8. In some experiments N-acetyl glucosamine, treated identically, was run simultaneously with the sialic acids.

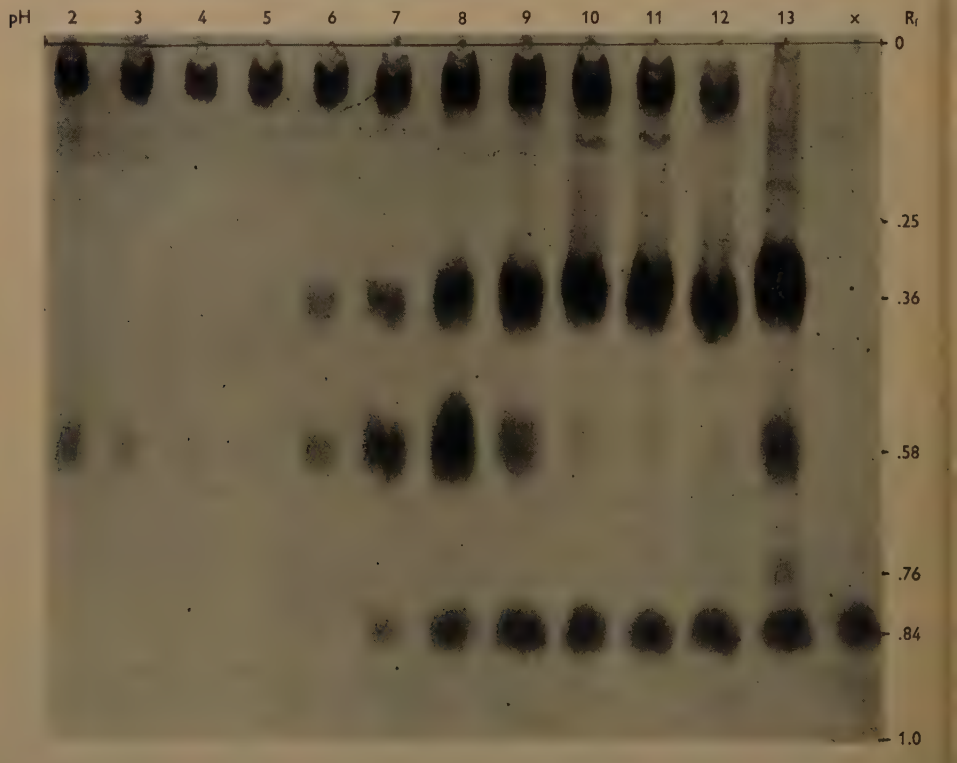

Fig. 4. Chromatogram of N-acetyl neuraminic acid after heating at 100°C for 1 hour in citrate–borate–phosphate–HCl buffer of pH 2 to 11, and in sodium hydroxide solution of pH 12 and 13. × denotes synthetic 2-carboxypyrrole. Solvent: BA. Spraying reagent: *p*-DAB.

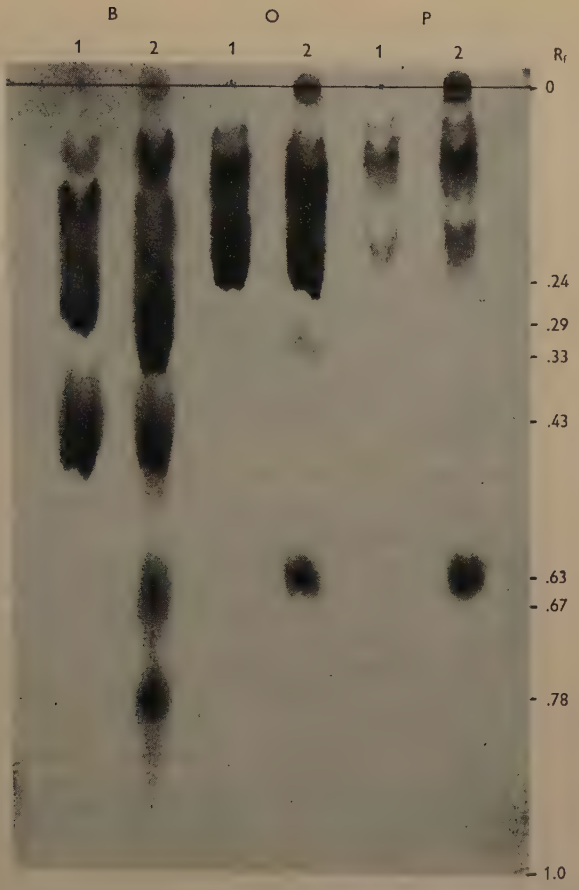

Fig. 5. Chromatogram of different sialic acids after heating in distilled water at 100°C for 1 hour. (B) N,O-diacetyl, (O) N-acetyl, and (P) N-glycolyl neuraminic acid. *1*, unheated solution; *2*, heated solution. Solvent: BA. Spraying reagent: *p*-DAB.

Heating of sialic acids under the conditions used regularly yielded chromatographically demonstrable products, not occurring in the unheated solutions. Besides these products some material was present in most experiments, which, to judge from controls with unheated sialic acid, contained undegraded substance, but possibly also some unidentified degradation products.

Heating in acid solution, pH < 4 (Figs. 4, 5). This treatment included heating for 1 hour in distilled water (pH of initial solution about 2.2), acid buffers, 1 *N* acetic acid, and a Dowex 50 suspension in water, at 100°C, and in 0.1 *N* sulphuric acid at 80°C.

N,O-diacetyl, N-acetyl, and N-glycolyl neuraminic acid heated in *distilled water* all gave rise to a spot with R_f 0.63 in BA. In addition other spots were also found with different R_f-values for each kind of sialic acid (Fig. 5). All the spots gave purple or violet colours with Ehrlich's reagent, and a reddish-purple with the orcinol reagent. They also reacted with aniline hydrogen phthalate giving a brown colour. The spots

did not appear after pretreatment (of the heated solution) with the anion exchange resin Dowex 1. Similar observations were made after chromatography in BP too.

The other forms of acid treatment gave essentially the same results. The nature of the acid agent used, however, seemed to influence the shape and, to some extent, the velocity of the spots, as well as their relative size. For that reason (and also depending on the amounts applied to the papers) one or other of the spots failed to appear in some runnings.

Heating at pH 4–5 gave rise to spots, the R_f of which in BA approximately corresponded to that of the previously mentioned spot with R_f 0.63. They were, however, much weaker than those formed at more acid conditions (Fig. 4).

Heating at pH >5 (Fig. 4). At pH 6 products were formed, moving in BA with R_f 0.36 and 0.58, respectively. At pH 7–13 a further one with R_f 0.84 appeared and at pH 12–13 (in 0.01 and 0.1 *N* sodium hydroxide) a faint spot with R_f 0.76. Finally, a weak spot, R_f 0.25, could sometimes be seen at alkaline pH. The relative sizes of the spots varied widely as seen in the figure. All products gave a purple colour with Ehrlich's reagent—some already at room temperature—which more or less rapidly changed to violet on prolonged heating. They also reacted with orcinol-trichloroacetic acid giving a reddish-purple colour.

The spot with R_f 0.84 in BA, coincided in colour shade and R_f with simultaneously run 2-carboxypyrrole (applied to the paper in water solution). The R_f-value of N-acetyl glucosamine in BA was 0.25, corresponding to the weak spot mentioned above.

In BP the spots formed were less distinct, often showing trailing and blurred boundaries. At the alkaline pH's spots occurred, corresponding to 2-carboxypyrrole and N-acetyl glucosamine.

Heating of sialic acids and N-acetyl glucosamine in alkaline solution of varying strength. Besides the products just described, additional ones were formed by heating in alkali under certain conditions. When treated with *0.04 N barium hydroxide at 90°C for 20 minutes*, sialic acids gave rise to spots with R_f in BA 0.25 (I), 0.37 (II), 0.45 (III), 0.58 (IV), and 0.83 (V) (Fig. 6*a*). Spot III, which gave a bright purple colour with the Ehrlich reagent instantaneously in the cold, was almost obscured by a more slowly developing forward trail as was spot IV. The three first spots reacted with the aniline reagent (Fig. 6*b*). Treatment with the resin Dowex 1 removed most of the spots, leaving only numbers I and III, and the trail in front of the latter (Fig. 6*c*).

From N-acetyl glucosamine, heated under similar conditions (0.02 *N* barium hydroxide, 90°C, 20 minutes) products were formed, wholly coinciding with the aforementioned spots I and III, the latter also with a forward trail, ending in a purple spot, R_f 0.83. None of these products was adsorbed by the resin.

In BP the chromatograms of the sialic acids and N-acetyl glucosamine were almost identical, except for the presence in the formers of larger amounts of material remaining on the starting line, and more marked purple trailing in front of it (Fig. 7). The R_f'-values of the distinct spots were 0.44 and 0.60, both violet with Ehrlich's reagent, and 0.69 and 0.79, both purple. The two former and the immobile material gave a brown colour with the aniline reagent.

By treatment with *0.04 N barium hydroxide at 100°C for 30 minutes* smaller amounts of those degradation products of sialic acids were obtained, which chromatographically corresponded to N-acetyl glucosamine and the chromogens deriving from this substance (Fig. 6).

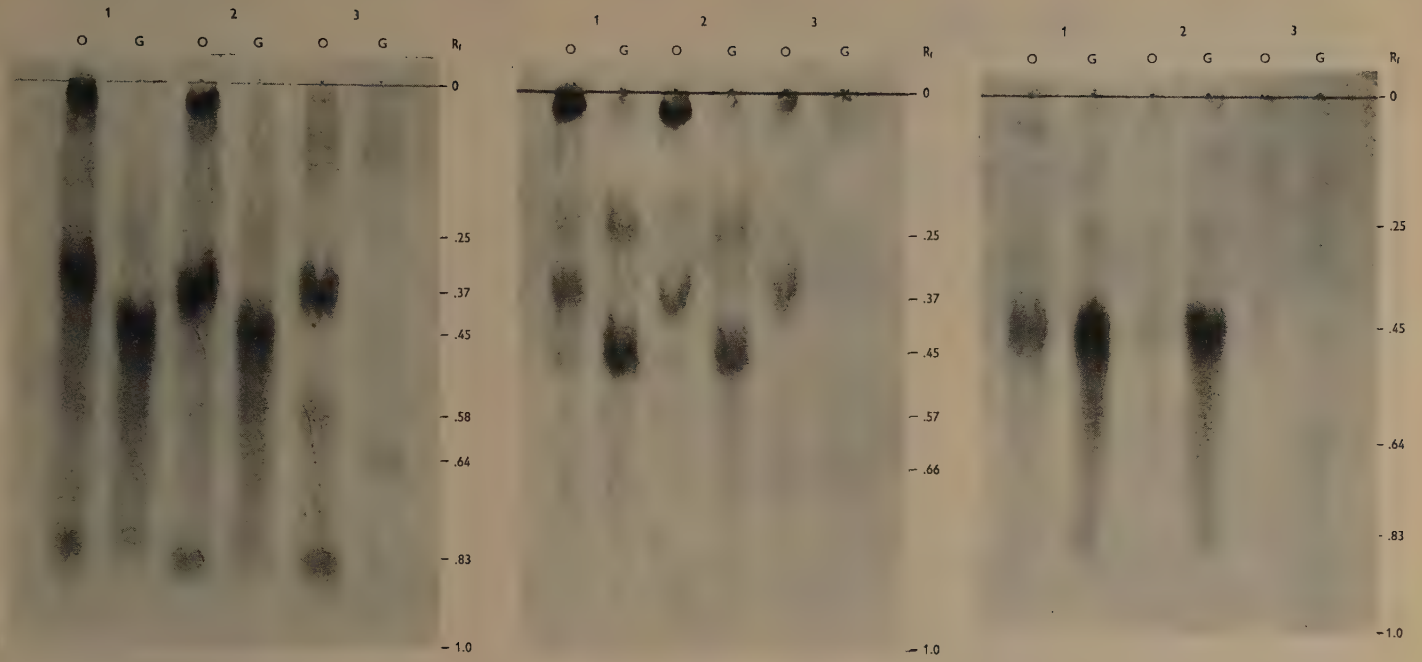

Fig. 6 a. Fig. 6 b. Fig. 6 c.

Fig. 6*a*. Chromatograms of N-acetyl neuraminic acid (O) and N-acetyl glucosamine (G) after heating in barium hydroxide solution. *1*, (O) 0.04 *N*, and (G) 0.02 *N* $Ba(OH)_2$, 90°C 20 minutes; *2*, same $Ba(OH)_2$ concentrations, 100°C, 30 minutes; *3*, 0.2 *N* $Ba(OH)_2$, 100°C, 30 minutes. Solvent: BA. Spraying reagent: *p*-DAB. Fig. 6*b*. See 6*a*. Spraying reagent: aniline hydrogen phthalate. Fig. 6*c*. See 6*a*. Solutions stirred with Dowex 1 before chromatography.

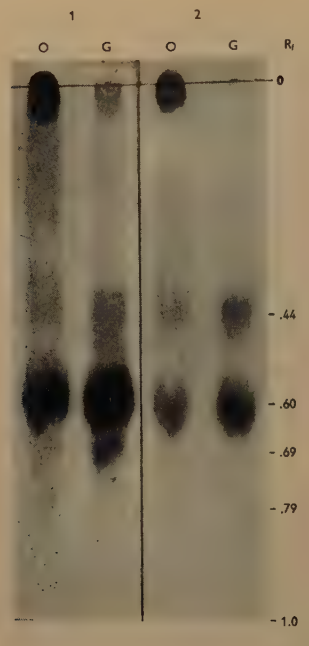

Fig. 7. Chromatograms of N-acetyl neuraminic acid (O) and N-acetyl glucosamine (G) after heating in (O) 0.04 *N* and (G) 0.02 *N* $Ba(OH)_2$, 90°C, 20 minutes. Solvent: BP. Spraying reagent: *1*, *p*-DAB; *2*, aniline hydrogen phthalate.

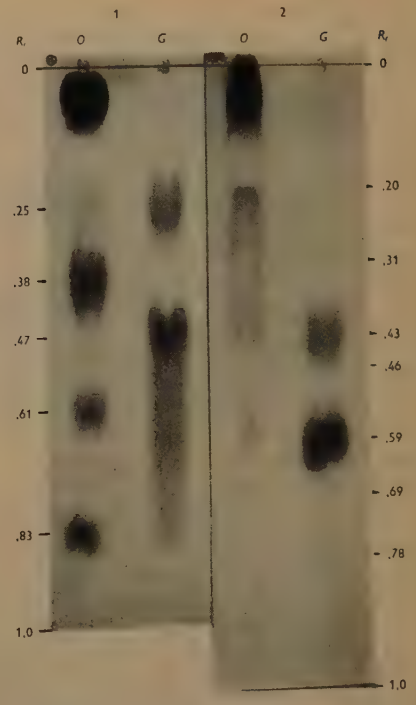

Fig. 8. Chromatograms of N-acetyl neuraminic acid (O) and N-acetyl glucosamine (G) after heating in phosphate buffer, pH 8, at 100°C for 1 hr. Solvents: *1*, BA; *2*, BP. Spraying reagent: *p*-DAB.

It was observed, that the pH of the solutions of sialic acid and N-acetyl glucosamine in 0.04 and 0.02 *N* barium hydroxide, respectively, had changed to neutral or even slightly acid after heating.

After treatment with *0.2 N barium hydroxide at 100°C for 30 minutes* none of these chromogens was found. N-acetyl glucosamine yielded only one distinct spot in BA, a weak purple one with R_f 0.64–66. The sialic acids gave rise to a corresponding spot together with the previously described products of alkaline treatment, not formed from N-acetyl hexosamine (Fig. 6).

Heating with *phosphate buffer pH 8, at 100°C for 1 hour*, seemed to produce only insignificant amounts of the "N-acetyl hexosamine chromogens" from sialic acids (Fig. 8).

Further alkaline treatment of chromatographically separated products. Some of the fractions obtained by heating the sialic acids in phosphate buffer, pH 8, were eluted separately, heated for 30 minutes in the same buffer, and rechromatographed. The slowest fraction, R_f 0.37, gave rise to two spots with R_f-values identical to those of the original two slower spots with R_f 0.37 and 0.58. The chromatographic behaviour of the products with R_f 0.58 and 0.84 was unchanged.

Ultraviolet absorption of the chromatographic fractions

N-glycolyl neuraminic acid was heated with 0.1 *N* sodium hydroxide for 30 minutes and chromatographed on paper. Sections corresponding to the three main spots (R_f 0.37, 0.58, and 0.84) and the trailing material were eluted with phosphate buffer, pH 8. All fractions had an ultraviolet absorption with a maximum at about 260 mμ, the eluates of the spots with R_f 0.37 and 0.84, showing high-intensity bands with maximal absorption at 263 and 257 mμ, respectively. The absorption of the slowly moving material, $R_f < 0.37$, represented 32 % of the total absorption of the eluates at 260 mμ, that of the 0.37-spot 45 %, the 0.58-spot 5 %, and the 0.84-spot 17 %.

Eluates of the product moving with R_f 0.63 in BA, obtained after heating N-glycolyl neuraminic acid in 1 *N* acetic acid, showed in this solvent a well-defined absorption peak with maximum at 272 mμ.

The absorption curves of synthetic 2-carboxypyrrole and of the eluate of the 0.84-spot both dissolved in phosphate buffer, pH 8, were nearly identical (Fig. 9).

The yield of 2-carboxypyrrole, obtained by heating a 0.02 *M* solution of N-glycolyl-neuraminic acid in 0.1 *N* sodium hydroxide for 30 minutes at 100°C was estimated by eluting the chromatographically separated fast-moving product (R_f 0.84) and comparing its absorption at 260 mμ with that of a known quantity of synthetic 2-carboxypyrrole, chromatographed and eluted in the same way. The yield of 2-carboxypyrrole was about three per cent of the theoretical.

Identification of the crystalline material isolated from N-glycolyl neuraminic acid methyl ester by alkali treatment

The X-ray powder diagram of the isolated material coincided with that of an authentic specimen of 2-carboxypyrrole as shown in Fig. 10.

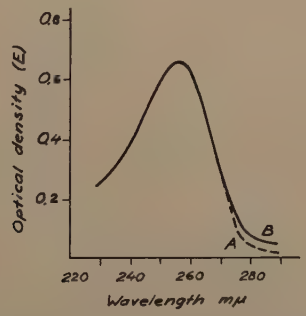

Fig. 9. Ultraviolet absorption spectra of (A) synthetic 2-carboxypyrrole, and (B) the eluted chromatographic fraction with R_f 0.84 in BA, obtained by alkaline degradation of N-glycolyl neuraminic acid. (Between 230 and 270 mμ the two curves are practically identical.)

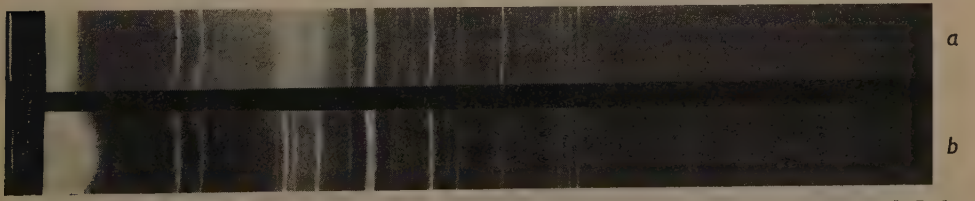

Fig. 10. X-ray powder diagrams of (a) the crystalline material isolated from alkali-treated N-glycolyl neuraminic acid methyl ester, and (b) synthetic 2-carboxypyrrole.

Discussion

The ring (I) and open-chain (II) structures of N-acetyl neuraminic acid as first proposed by Gottschalk (15) are depicted in Fig. 11, using the configuration assumed for "lactaminic acid" by Kuhn and Brossmer (16).

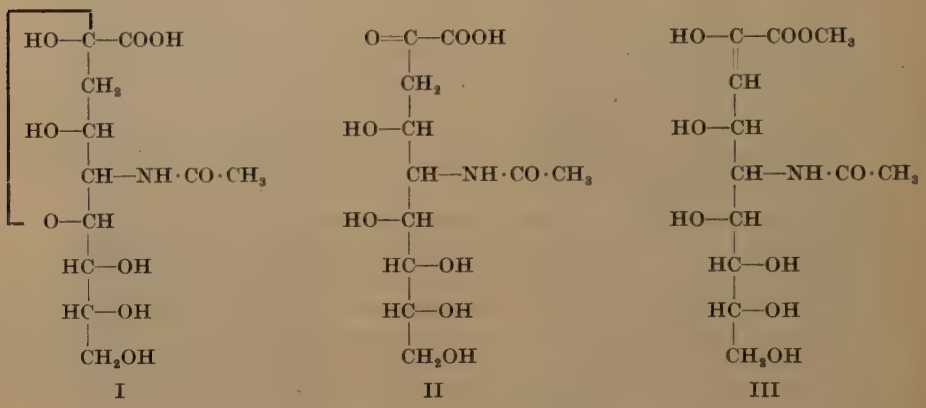

Fig. 11.

Periodate-oxidation experiments (7) and infrared absorption data (17) indicate a ring structure of the sialic acids in water solution and in the solid state, respectively. The ultraviolet absorption spectra in aqueous solution do not give any information as to whether the free acids occur in the ring or in the open-chain form. (The carbonyl form cannot be expected to show an ultraviolet absorption—in the 280 mμ region—forming a 2-unit conjugated system, terminated by two oxygen atoms.)

The well-defined, high intensity, transient absorption with a maximum at about 265 mμ, given by the sialic acid methyl esters in neutral and moderately alkaline solution at room temperature, is interpreted to indicate a 2-unit conjugated system, the formation of which may be explained by an enolization (III) of the open-chain carbonyl form. The disappearance of this absorption band is in all probability due to hydrolysis of the ester. The fact that the transient absorption could not be demonstrated in more alkaline solution, such as 0.1 *N* sodium hydroxide, is probably due to a momentary or very rapid saponification. It is known that the sialic acid methyl esters are easily hydrolyzed even at very mild conditions (18).

There is no obvious explanation of the slower decrease of the absorption at pH 9–11 as compared to that at pH 8. This fact, however, in our opinion does not invalidate the interpretation of the transient absorption given above.

The absence of distinct absorption bands in solutions of non-esterified sialic acids may be explained by the assumption that enol forms of these substances are formed only in insignificant amounts at the conditions used. The ester group should then favour the enolization of sialic acids in neutral and alkaline solution. A similar effect of esterification has been demonstrated for acetoacetic acid, the ethyl ester of which in alkaline solution shows 20 times higher ultraviolet absorption than the free acid, and which has been reported to be enolized to 100 % against about 5 % for the acid (19). The absorption of the acetoacetic ester also gradually decreases, which was interpreted as due to saponification.

By heating in acid solution the sialic acids are decomposed with humin formation. Several chromatographically separable degradation products are formed, reacting with the Ehrlich, orcinol, and aniline reagents. The products seem to have acid properties, as they are adsorbed by an anion exchanger. One of them is produced by all the three kinds of sialic acid tested. The absorption in ultraviolet—max. 270 mμ—occurring after acid treatment is due, at least partly, to this product. It can accordingly be assumed to be a—probably cyclic—compound, containing 2 or 3 conjugated double bonds.

As the procedures used for the isolation of sialic acids from various materials include heating at acid conditions, it is very probable, that at least a small part of the substance will always be degraded. This fact may explain the observations made (8, 18, 20) that, when acid hydrolysates of sialic acid-containing materials are eluted from anion exchangers, besides the crystallizing sialic acid a small non-crystallizing fraction giving the same colour reactions is obtained. Yamashina (20) suggested from such findings the presence of two types of sialic acid in the α_1-acid glycoprotein of plasma. He also found, however, that the column fraction, corresponding to N-acetyl neuraminic acid, by continued acid treatment could be largely converted to the second fraction. This observation clearly indicates a partial decomposition of an originally present sialic acid, taking place during treatment with acid.

Heating at pH 4–5 for several hours gives no absorption bands and, chromatographically, only small amounts of degradation products are formed. The maximum stability of sialic acids in aqueous solution thus seems to be at pH about 4.5.

After treatment of sialic acids at mild alkaline conditions Kuhn and Brossmer (6) and Zilliken and Glick (5) were able to isolate N-acetyl-D-glucosamine as a degradation product. Studying the mechanism of the Morgan–Elson reaction, Kuhn and Krüger (21, 22) have found that three chromatographically demonstrable chromogens are formed from N-acetyl hexosamine by mild alkaline treatment. One of these, chromogen I, seemed to be an anhydrohexosamine; another, chromogen III, was identified as 5-dihydroxyethyl-3-acetaminofuran.

In the present investigation it was found that by heating sialic acids with very dilute alkali, besides free N-acetyl hexosamine, three Ehrlich-positive chromogens were formed, chromatographically coinciding with those produced by N-acetyl glucosamine treated under the same conditions. These products were found to be rapidly destroyed by stronger treatment with alkali.

Gottschalk has prepared a dialysable product from bovine submaxillary mucin treated with alkali under very mild conditions (23). He suggested that the product was a disaccharide, composed of N-acetyl neuraminic acid and N-acetyl galactosamine (or rather that it formed a disaccharide-chromogen, being composed of the former substance linked to an alkali degradation product of the latter). The assumption was based on the observation, that the product on treatment with influenza virus gave rise to (1) N-acetyl neuraminic acid and (2) a chromogen, which chromatographically behaved like a product derived from N-acetyl hexosamine, treated with weak alkali, and which is probably identical to chromogen I of Kuhn and Krüger. Treatment with stronger alkali resulted in the formation of (1) 2-carboxypyrrole and (2) a substance—distinct from chromogen I—which was also formed by alkali degradation of N-acetyl hexosamine. Gottschalk's suggestion may be quite correct, the evidence, however, seems inconclusive, as sialic acid itself under the appropriate conditions gives rise to N-acetyl hexosamine — and consequently also to chromogens deriving from this substance.

Besides the products just described the alkali-treated sialic acids give rise to 3 or 4 further decomposition products. All of these give purple and violet colours with the Ehrlich and orcinol reagents and show an ultraviolet absorption at about 260 mμ. The nature of the products running with R_f 0.37 and 0.58, respectively, in butanol–acetic acid, is not known. Both react with the aniline reagent and show acid properties. The ultraviolet absorption indicates structures containing 2 or 3 conjugated double bonds, and the reaction with aniline possibly furan derivatives. The slower-running product seems to be at least partly converted into the faster one on continued alkali treatment. None of them gives rise to 2-carboxypyrrole by this treatment.

Gottschalk presented strong evidence (3, 4) that the fastest-running fraction in butanol–acetic acid is 2-carboxypyrrole. Its identity with this compound has now been definitely established by isolation of the product in crystalline form from sialic acid methyl ester and its identification by X-ray analysis.

The yield of 2-carboxypyrrole is very low—about three per cent of the theoretical after heating in 0.1 *N* sodium hydroxide for half an hour. Less than 20 % of the ultraviolet absorption at 260 mμ is due to the formation of 2-carboxypyrrole at these conditions. The ultraviolet absorption arising in alkali-treated sialic acid solutions can therefore obviously not be used for the determination of the 2-carboxypyrrole formed. This seems to have been overlooked in some instances (24).

SUMMARY

Sialic acids, heated in aqueous solution of different pH, show a stability maximum at pH 4 to 5. At more acid or alkaline conditions they are degraded by comparatively mild treatment, as demonstrated by ultraviolet and chromatographic examination. One of the alkali degradation products, 2-carboxypyrrole, was isolated and identified by X-ray analysis. Among the further products of alkaline treatment the formation of N-acetyl hexosamine and some chromogens derived from this substance was indicated. A transient ultraviolet absorption, occurring on dissolving sialic acid methyl ester at neutral and moderately alkaline reaction, was observed. It was interpreted as due to enol formation from the open-chain keto form of the substance.

Our thanks are due to Miss Ulla Lindberg for most valuable technical assistance.

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Tryckt den 11 augusti 1958

Uppsala 1958. Almqvist & Wiksells Boktryckeri AB